Antibiogram Patterns of *Staphylococcus aureus* Obtained from Packaged Meat, Milk and Milk Products, Fruits and Vegetables of Various Supermarkets in Dhaka City

A DISSERTATION SUBMITTED TO BRAC UNIVERSITY IN PARTIAL FULLFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF BACHELOR OF SCIENCE IN BIOTECHNOLOGY

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Dedicated to all my loved ones and

my supervisor

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DECLARATION BY THE RESEARCHER

This is to declare that the research work embodying the results reported in this thesis work entitled **"Antibiogram Patterns of *Staphylococcus aureus* Obtained from Packaged Meat, Milk and Milk Products, Fruits and Vegetables of Various Supermarkets in Dhaka City"** submitted by the undersigned has been carried out under the supervision of Zubaida Marufee Islam, Lecturer, Biotechnology program, Department of Mathematics and Natural Sciences, BRAC University. It is further declared that the research work presented here is original and submitted in the partial fulfilment for the degree of Bachelors of Science in Biotechnology, BRAC University, Dhaka and has not been submitted anywhere else for a degree or diploma.

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ABSTRACT

Staphylococcus aureus is the third most common food borne pathogen in the world. It is highly virulent and produces 20 different types of enterotoxins and it is recently being noticed that *Staphylococcus aureus* isolated from food is also developing antibiotic resistance. The purpose of this study was to isolate and identify *Staphylococcus aureus* from frequently bought food items from supermarkets around Dhaka. The samples are divided into three categories of food: milk and their products, packaged meat, fruits and vegetables. Thirty samples of these food groups were collected from three different supermarkets, most popular ones, around Dhaka and transported to the lab in sterile conditions. The extract was collect and organism isolated using selective medium Mannitol Salt Agar, MSA (HiMedia). The suspected colonies (16 isolates) were subjected to several biochemical tests to verify its identity. Positive samples were then subjected to an antibiotic sensitivity test. Following antibiotics were used: Nalidixic acid(30µg), Rifampicin(5µg), Tetracycline(30µg), Gentamicin(10µg), Amoxycillin(10µg), Co-trimoxazole(25µg), Novobiocin(30µg), Levofloxacin(5µg), Chloramphenicol(30µg), Oxacillin(1µg), Erythromycin(1µg), Ampicillin(10µg). More than half the samples isolated contained *Staphylococcus aureus* and some contained *Staphylococcus epidermidis* suggesting a public health concern due to contamination of food products by pathogenic bacteria. This contamination is a result of unhygienic processing, handling and storage in supermarkets.

Keywords: *Staphylococcus aureus*, antibiogram, meat, milk, fruits and vegetables, food poisoning, supermarkets

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LIST OF ABBREVIATIONS

Abbreviation	Full form
S. aureus	***Staphylococcus aureus***
S. epidermidis	***Staphylococcus epidermidis***
MRSA	**Methicillin Resistant *Staphylococcus aureus***
MSSA	**Methicillin Sensitive *Staphylococcus aureus***
FBD	**Food Borne Disease**
SE	**Staphylococcal Enterotoxin**
SFP	**Staphylococcal food poisoning**
NA	**Nutrient Agar**
MSA	**Mannitol Salt Agar**
MHA	**Mueller-Hinton-Agar**
NaCl	**Sodium Chloride**
H_2O_2	**Hydrogen peroxide**
CO_2	**Carbon dioxide**
TSI	**Triple Sugar Iron**
MIU	**Motility, Indole, Urease**
KOH	**Potassium Hydroxide**
MR	**Methyl Red**
VP	**Vogues Proskaeur**

1. INTRODUCTION

1.1 Food Borne Disease (FBD):

According to the World Health Organization, a Food Borne Disease (FBD) is a disease of infectious or toxic nature that is caused by the consumption of contaminated food or water. Millions of people worldwide are suffering from communicable and non-communicable food borne diseases which create a significant impact on human lives, especially among children and the elderly (Van der Vanter, 1999). Food borne diseases are widespread throughout both developing and developed countries and its prevalence not only affects the health of an individual but has serious repercussions on his economic situation as well as that of the country (Carbas *et al,* 2012). As such, it is of utmost importance that the incidences of food poisoning are under surveillance. However, a country like Bangladesh has no epidemiological surveillance system and therefore it is very difficult to estimate the number of cases.

Among the most common food-borne pathogen is *Staphylococcus aureus*. Usually the pathogenesis of food borne pathogens takes either of two paths. The pathogen may release toxins in the digestive tract of the individual who consumed the contaminated food product or the person may consume food with preformed enterotoxins (intoxication). *S. aureus* follows the second method and according to the works is the leading cause of gastroenteritis from the consumption of tainted food (Loir *et al,* 2003).

1.2 *Staphylococcus aureus*:

S. aureus is a Gram-positive coccus that appears as grape-like ('Staphylo' means grape in Greek) clusters when viewed under a microscope (Loir *et al*, 2003). When streaked across nutrient agar, it produces colonies that are smooth, circular and convex and may be 0.5-1.5μm in diameter.

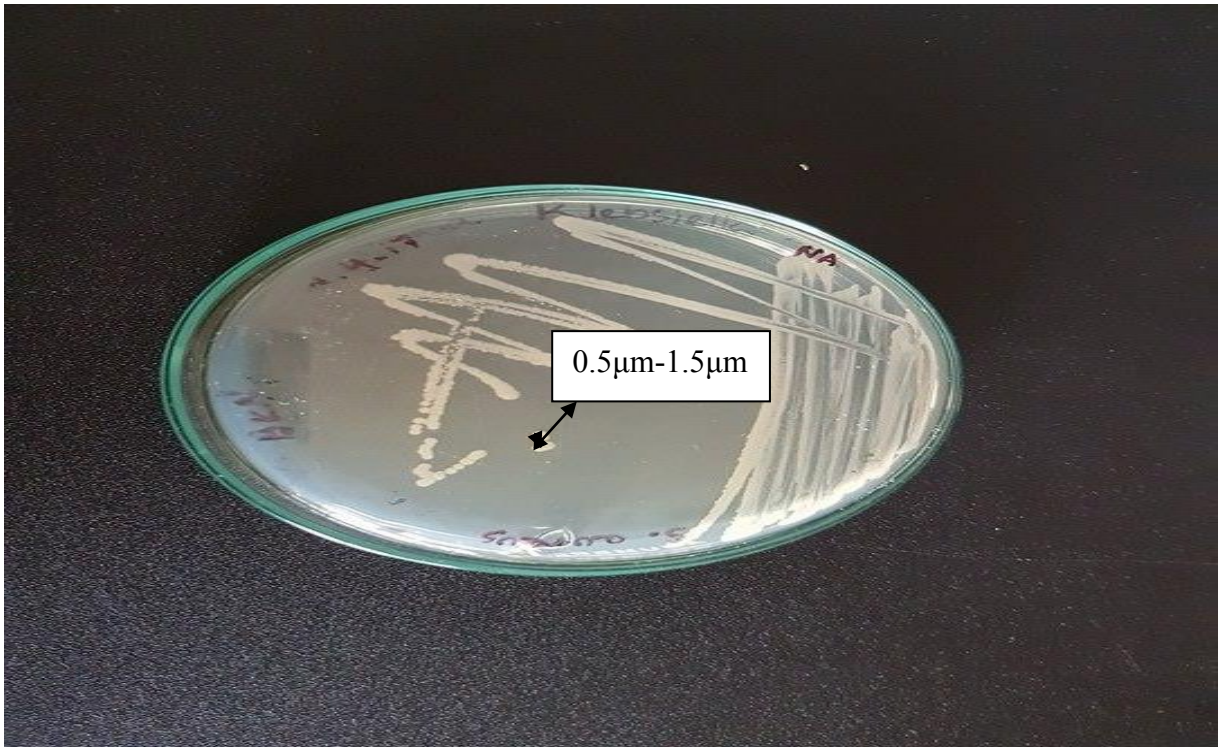

Figure 1: *S. aureus* streaked on Nutrient Agar (NA) to show single colony

The pigmentation of the colonies may vary from cream colored, golden yellow to orange shades depending on where it is isolated from. Thus earning the name 'aureus' which is latin for 'golden' (Jahan *et al*., 2015).

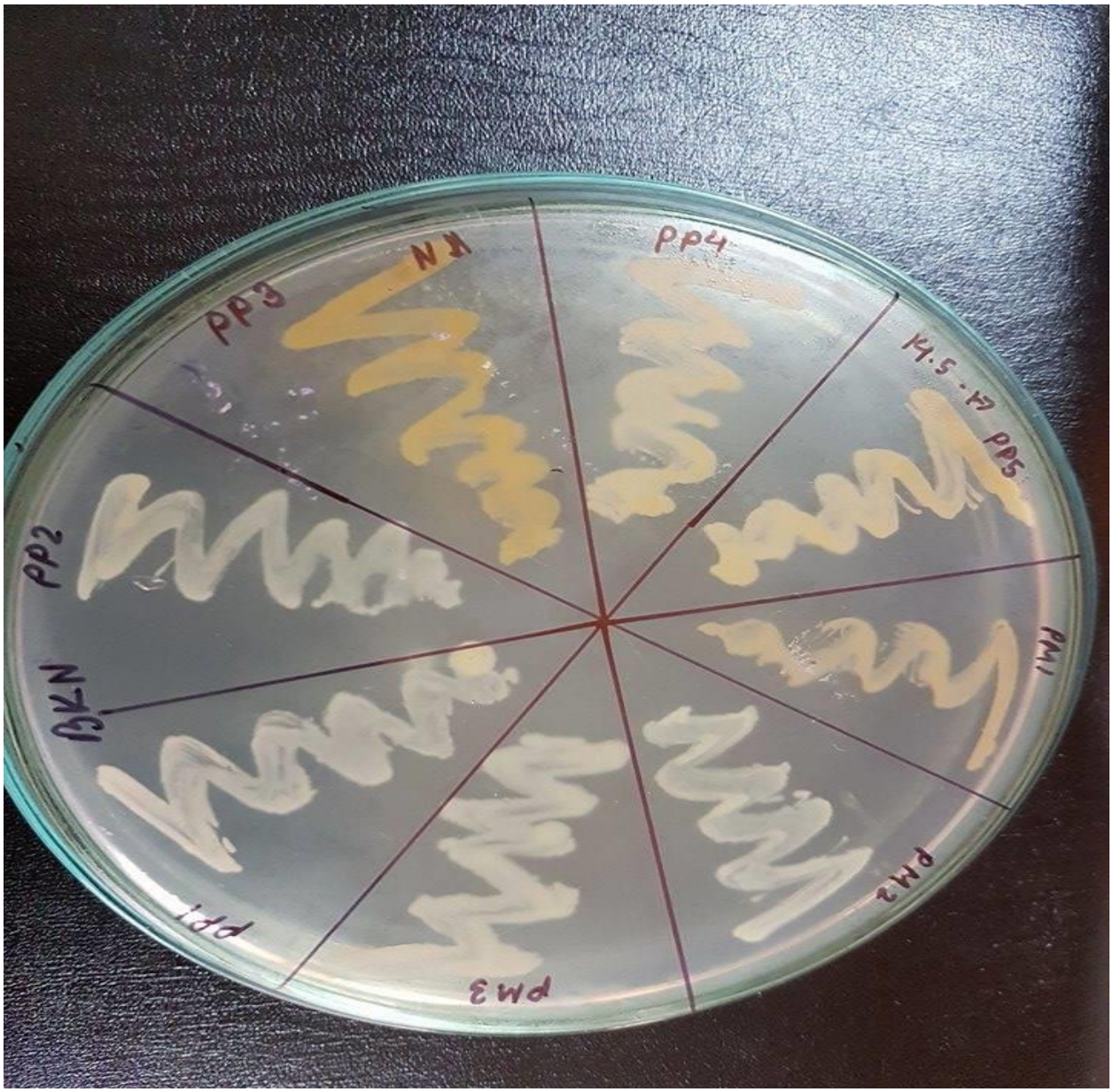

Figure 2: Samples of *S. aureus* streaked on Nutrient agar showing varying levels of pigmentation

S. aureus is found in the nasal passages, skin and hair of warm blooded mammal but may also be isolated from various foodstuffs such as meat, dairy, cheese as well as the environment (Kools and Schleifer, 1986). Up to 30-50 % of the human populations are carriers. *S. aureus* is able to survive in a wide range of temperatures, ranging from 7°C to 48.5°C; with an optimum temperature of 30°C to 37° C (Schmitt *et al*, 1990), pH ranging from 4.2 to 9.3; with an optimum pH of 7 to 7.5; (Bergdoll, 1983) and sodium chloride concentrations of up to 15%. The ability to

survive in various temperatures and pH allows *S. aureus* to infect several types of food products (Loir *et al*, 2003).

1.3 Staphylococcal food poisoning:

Staphylococcal food poisoning includes symptoms such as nausea, vomiting, abdominal cramps and diarrhea (Balaban and Rasooly, 2000). *S aureus* does not produce endospore (Loir *et al*, 2003) and thus is destroyed upon heating at a normal cooking temperature. However, the enterotoxins remain active (Prescott *et al*, 2002). The enterotoxins are highly heat resistant (Bergdoll, 1983). More than 50% of the strains produce enterotoxins making it quite dangerous for an individual to consume a food product contaminated with *S. aureus (*Payne and Wood, 1974). *S. aureus* causes a wide range of disease, starting from mild ones such as boils, pimples, abscesses, cellulites, toxic shock syndrome, impetigo and proceeding to more life-threatening ones such as pneumonia, meningitis, endocarditis, septicaemia (Soormo *et al*, 2003). In humid countries like Bangladesh, the rate of infection is much higher, specifically in food as most storage facilities are unhygienic. Therefore, the purpose of this study was to isolate and identify *S. aureus* from three food groups commonly bought in popular supermarkets.

1.4 Contamination of food by *S. aureus*:

Supermarkets are very convenient in this fast-paced world as several things are found at the same place. However, it is quite necessary to make storage hygienic to ensure minimal contamination of food groups such as milk and its products, packaged meat, fruits and vegetables. These have highest chance of contamination as they are usually processed a lot and require a lot of handling and distribution.

In most Staphylococcal food poisoning cases, the food in question or one of its ingredients was supposedly exposed to a stain of *S. aureus* that is capable of producing Staphylococcal enterotoxin (SE) upon exposure to a temperature that allows growth of *S. aureus* (Loir *et al*, 2003). In fact, for a Staphylococcal food poisoning (SFP) outbreak to occur; five conditions need to be present (Asao *et al*, 2003) and (Pereira *et al*, 1996):

1. A raw material or an infected person containing an enterotoxin producing *S. aureus* species.

2. Transfer of the bacteria from source to food via unhygienic preparation.

3. Food must have favorable physiochemical properties to support *S. aureus* growth and toxinogenesis.

4. Sufficient time for bacterial growth and toxin production.

5. And finally, ingestion of contaminated food product.

While it is known that improper handling and cooking enables growth, also notes that inadequate cooling of foods can also induce Staphylococcal growth/toxin production, resulting in food poisoning (Barber, 1914).

Several types of food can provide a medium of growth for *S. aureus* milk, cream, butter, sandwich fillings, ham, cheese, sausages, canned meat, salads, and vegetables (Loir *et al*, 2003). Several cases have been recorded. One case was the contamination of cheese where the milk used was contaminated after pasteurization and before addition of lactic starter culture. Thus starter culture did not grow properly enabling growth of *S. aureus* and production of SEs. Another case occurred in 1985 in Kentucky, USA. The food in question was chocolate milk which was contaminated before pasteurization. The pasteurization process killed the *S. aureus* but the SEs remained. Such examples emphasize the importance of uncontaminated raw materials and refrigeration of food to prevent bacterial growth (Bergdoll, 1983).

1.5 Staphylococcal prevalence in meat, milk and fresh produce:

As part of this project, three prominent types of food were evaluated. Each had a different medium to allow growth of *S. aureus* and provide favorable conditions for production of enterotoxins.

Milk: Due to its high nutritive value and complex chemical composition, it provides a favorable medium for several microorganisms to grow (Soomro *et al*, 2003). Milk and its derivatives are a major source of *S. aureus* infection in man (Zecconi and Piccinini, 1998). Milk extracted from *S. aureus* infected cows (mastitis) also contaminates milk (Akbar and Anal, 2013).

Meat: Poultry meat is a prominent carrier of food borne pathogens followed by red meat (Hughes *et al*, 2007). *S. aureus* growth is particularly favored in fresh and ready to eat (packaged and canned) meat (Aydin *et al*, 2011).

Vegetables, fruits and their juices: The samples are minimally processed ready to eat vegetables or fruits, or freshly squeezed fruits are being increasingly sold in supermarkets. However, these samples are still handled by workers whose hands may be contaminated during slicing or shredding of the product in question. This contact increases chance of contamination (El-Hadedy and El-Nour, 2012).

1.6 Antibiotic resistance of *S. aureus:*

The increased resistance to antibiotics among food borne pathogens is due to its continual use in human therapy. Drug resistant bacteria such as methicillin resistant *Staphylococcus aureus* (MRSA) are much more difficult to treat than methicillin sensitive *Staphylococcus aureus* (MSSA), (Akbar and Anal, 2013). It is essential to monitor the prevalence of antibiotic resistance in food borne pathogens to ensure food safety planning and to intervene in targeted parts of a procedure which might lead to food contamination (Fernández *et al*, 2012). Food plays an important part in the transfer of antibiotic resistance genes from food microflora to pathogenic organisms (Pereira et al, 1996). In a country like Bangladesh, where antibiotics are available without a prescription this is a very severe issue. Therefore, a part of this project was to create an antibiogram profile to evaluate the antibiotic resistance or sensitivity for each positive sample of *S. aureus* with 12 antibiotics.

1.7 Rationale of the research:

Currently there is a lack of data concerning the safety and hygiene of food stored at a supermarket. This may be due to prior assumptions that the food stored is hygienic as compared to street food. This study will help contribute to the absence of data of the contamination of food products stored in a supermarket by *S. aureus*.

1.8 Objectives of the study:

Increasing number of people prefers to shop in supermarkets as it provides a convenient place to shop for several goods at the same time. However, since it is already assumed that storage conditions are efficient, no thought for improvement is made. In a country like Bangladesh where education is lacking, people are not quite aware of the regulations that must be followed to prevent contamination or regulations are simply disregarded specifically *S. aureus* as it is present in mammalian skin. It is also notable to know that most people are unaware of how antibiotics

work. Sadly, several doctors in this country are unaware as well and prescribe the use of antibiotics for several diseases which are not caused by bacteria. It is expected that the contents of the study may promote research for further monitoring of storage conditions within supermarkets and raw materials used by manufacturers and help raise awareness of the dangers of antibiotic abuse.

Therefore, the objectives of this research were:

- Isolation and identification of *S. aureus* from samples of meat, milk and its products, fruits and vegetables are collected from three supermarkets (Agora, Shwapno, Meena Bazaar).
- Confirmation of *S. aureus* with selected biochemical tests.
- Testing antibiotic sensitivity against the positive *S. aureus* isolates.

2. METHODS AND MATERIALS

2.1 Collection of samples:

30 samples were collected from three supermarkets across Dhaka. Each sample was collected and refrigerated in its packaging to ensure least chances of contamination. Vegetables were collected and kept in sealed Ziploc bags and refrigerated to prevent contamination. All samples were tested in BRACU MNS laboratory.

Table 1: List of samples collected from three supermarkets

Sample number	Sample name
Sample 1(S1)	Aarong Sweet Yoghurt
Sample 2 (S2)	Aarong Butter
Sample 3(S3)	Paneer
Sample 4(S4)	Aarong Laban
Sample 5(S5)	Aarong Sour Curd
Sample 6(S6)	AarongBorhani
Sample 7(S7)	Aarong UHT Chocolate milk
Sample 8(S8)	Canned Chicken Meat
Sample 9(S9)	Blue cheese
Sample10(10)	Store Bought salad(sealed)
Sample 11(S11)	Conserved fruit
Sample 12(S12)	Mozarella
Sample 13(S13)	Raw Tangerine juice
Sample 14(S14)	Shakti doi (yoghurt)
Sample 15(S15)	Pran UHT Milk
Sample 16(S16)	Aarong UHT Strawberry Yoghurt
Sample 17(S17)	Lettuce
Sample 18(S18)	Spinach
Sampe19(S19)	Packaged Beef Bacon
Sample 20(S20)	Aarong Ghee(Clarified Butter)
Sample 21(S21)	Strawberry
Sample 22(S22)	Packaged Smoked Chicken

Sample 23(S23)	Milk Vita
Sample 24(S24)	Cauliflower
Sample 25(S25)	Mayonnaise
Sample 26(S26)	Raw Milk
Sample 27(S27)	Brie
Sample 28(S28)	PranLassi
Sample 29(S29)	Cucumber
Sample 30(S30)	Packaged sausages

Table 2: Collected samples categorized into separate food groups

Sample Type	**Number of Samples**
Fruits and Vegetables	8
Packaged Meat	4
Milk and its Products	18

2.2 Isolation of *S. aureus*:

The isolation procedure was similar to Singh and Prakash, 2008 with slight modifications. 5g of each sample was taken in a sterile Ziploc bag and to it 50ml of 0.1% peptone salt solution was added and homogenized using a mortar and pestle.

If sample was liquid, direct extract was used, homogenization was not required. Enrichment broth used was 0.9% saline. 5.6 ml or 5.6 g of sample extract was collected and placed in 100 ml conical flask and to it 50 ml of 0.9% saline solution was added. The samples were placed in an incubator for 24 hours at 37°C.

All equipment necessary was washed with tap water and then distilled water and certain tools were sterilized with 70% ethanol.

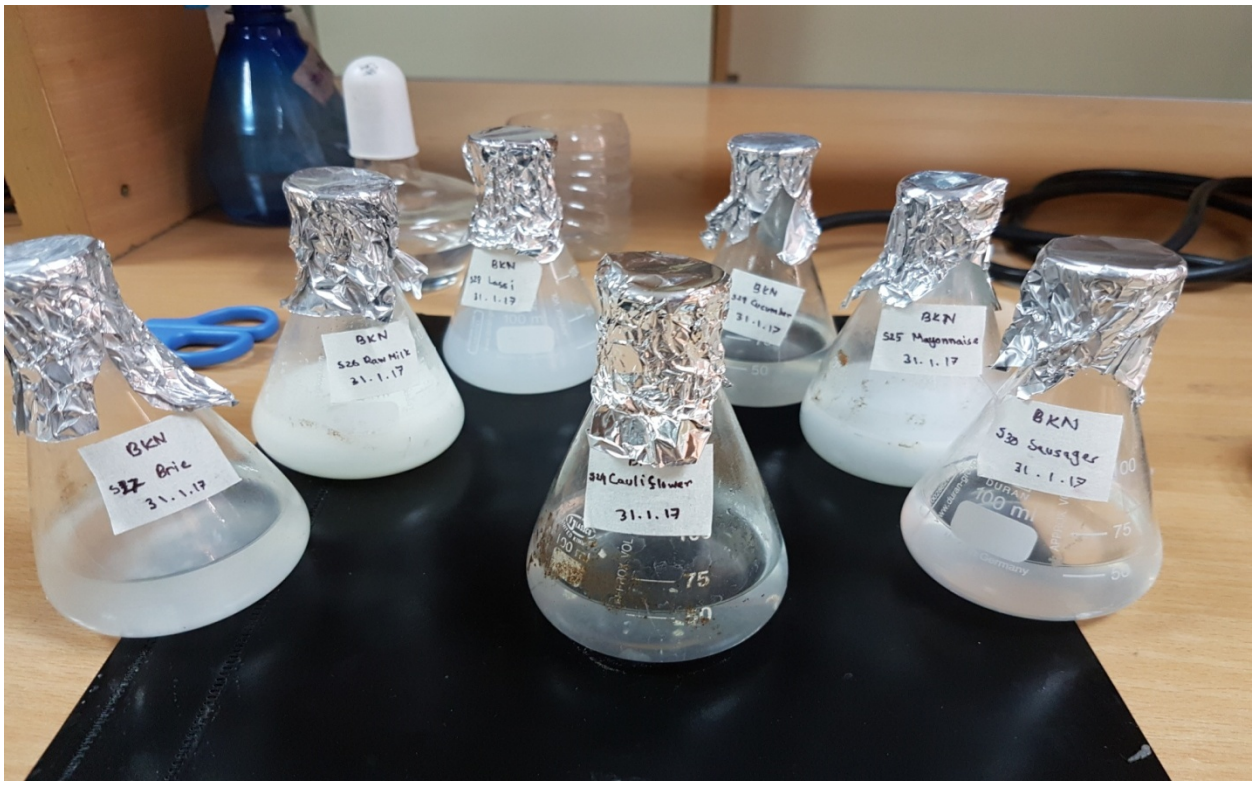

Figure 3: Samples in 0.9% saline solution to be incubated and enriched(Labeled from top row left side, A= S27, B= S24, C=S30, D=S26, E= S25, F=S28, G=S29)

After 24 hours, the samples were taken from the incubator and placed in a laminar flow cabinet. The samples underwent spread plating technique to separate the colonies. This was needed as the selective media used was Mannitol Salt Agar (MSA), HiMedia, which is selective for both *S. aureus* and *Staphylococcus epidermidis*. Therefore, it was needed to separate out the colonies to obtain pure culture of *S. aureus*. The colonies were spread on Nutrient Agar (NA), Oxoid. 100µl of extract was taken in a sterile microcentrifuge tube and to it 900µl of 0.9 % saline solution was added. The solution was vortexed to create a dilution solution of 10^{-1}. 100µl of the dilute solution was transferred to another microcentrifuge tube and to it 900µl saline was added and continued until dilution factor of 10^{-4} was reached. Each solution was streaked on separate NA plate using sterile glass rod and cultures incubated at 37° for 24 hours.

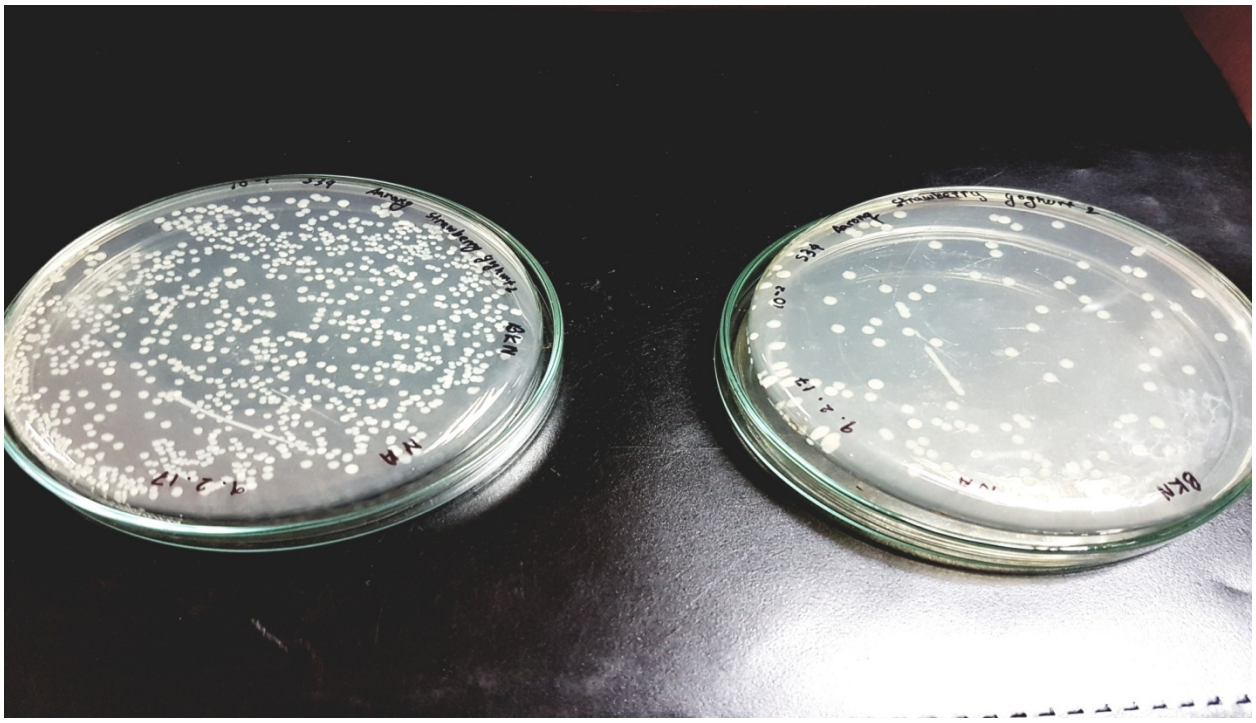

Figure 4: Sample that has been spread on NA plate showing 10^{-2} and 10^{-3} dilution factor

The following day each colony was separated and streaked on NA plates with each sample bearing the name S1, S2… and each colony separated from each sample bearing name O1, O2…. The samples were further incubated for 24 hours in 37°C. After incubation, each organism of each sample was streaked on MSA and again incubated for a further 24 hours at 37°C. After final incubation, pure colonies of *S. aureus* were obtained on MSA which appeared as yellow colonies. *S. epidermidis* appeared as pink colonies.

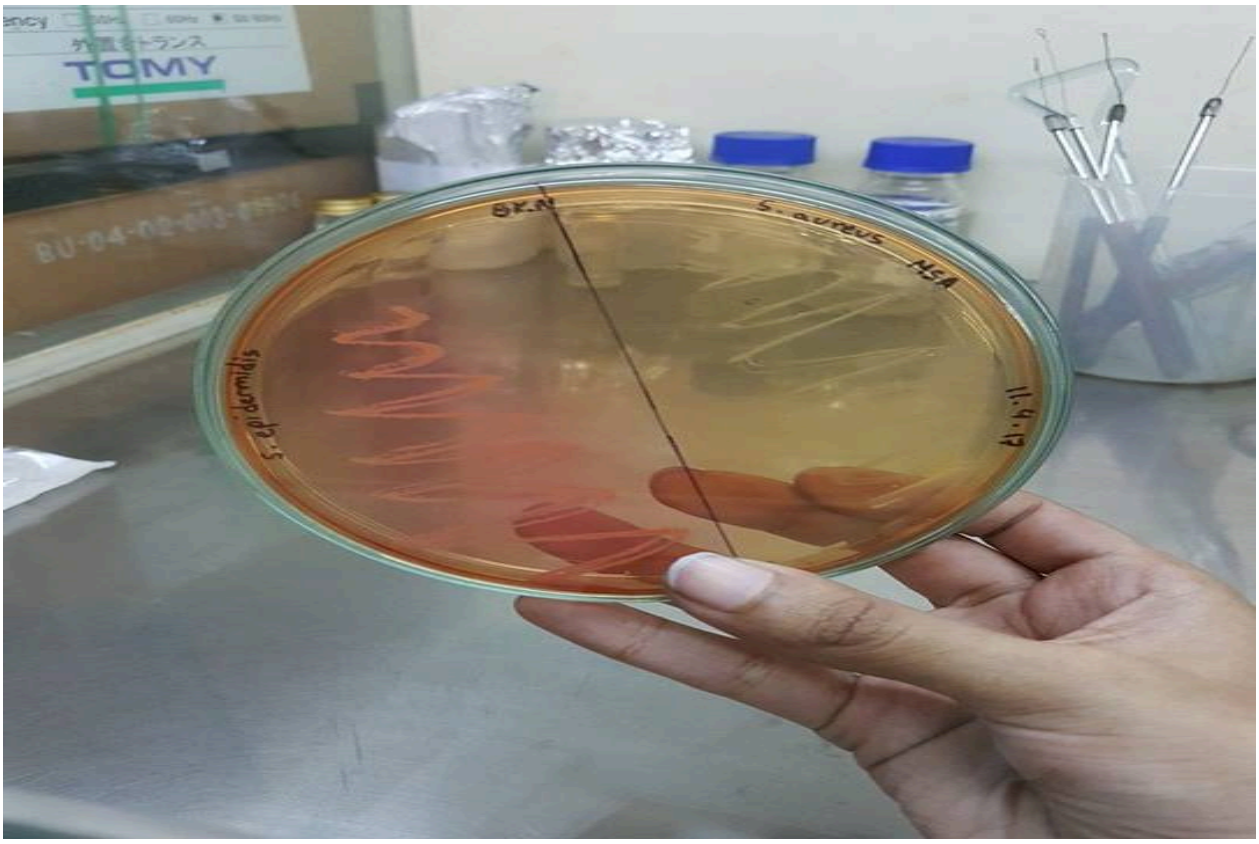

Figure 5: *S. aureus* (yellow) on right and *S. epidermidis*(pink) on left on MSA

The following day the positive samples were sub cultured onto NA plates and incubated at 37°C for 24 hours and refrigerated for storage. 16 suspected colonies were deemed positive and renamed according to their food group.

Table 3: List of suspected positive colonies categorized into food groups with new sample number

Original sample number	New sample number	Sample type
S10O1	PP1	**Fruits and Vegetables**
S13O4	PP2	
S17O2	PP3	
S24O1	PP4	
S29O1	PP5	
S8O1	PM1	**Meat**
S30O2	PM2	

S22O1	PM3	
S19O2	PM4	
S9O2	PY1	**Milk and Products**
S14O3	PY2	
S26O4	PY3	
S5O1	PY4	
S6O2	PY5	
S7O2	PY6	
S4O1	PY7	
***PP = fruits and vegetables, *PM = meat, PY*=milk**		

2.3 Identification of *S. aureus*:

Several biochemical tests were performed for the confirmation and identification of the 16 isolated colonies.

2.3.1 Citrate Utilization test:

The citrate utilization text is used to test an organism's ability to metabolize citrate as a source of energy. The medium is a mixture of citrate as the only energy source and an inorganic ammonium salt ($NH_4H_2PO_4$)as the only nitrogen source. The medium also contains bromothymol blue. Bacteria that can grow on citrate agar contain an enzyme known as citrate permease which can convert citrate to pyruvate which can enter into the Krebs cycle for energy production. Upon metabolization of citrate (an intermediate metabolite in Krebs cycle) the ammonium salts are broken down to ammonia which increases alkalinity. The increase in pH causes the bromothymol indicator in medium to turn from green to blue (above 7.6). Therefore, indicating that an organism is citrate positive.

Before sterilization, the media was distributed, 2ml each, into vials. After sterilization, the vials were tilted to create a slant and allowed to harden and then stored. A fresh subculture of the suspected, positive colonies was streaked on the citrate agar using a sterile inoculating loop or needle and incubated for 24 hours at 37°C. The next day results were recorded.

The citrate agar has certain limitations:

1. Some organisms are capable of growth without colour change. Presence of growth is indicative of a positive result.
2. This reaction alone does not produce the results for identification of a particular species.
3. Uncertain results may arise which need to be repeated.

2.3.2 Methyl Red(MR) test:

Some bacteria are able to metabolize glucose and form a structurally stable acid such as lactic, acetic or formic acid. The dextrose is first converted to pyruvic acid which is further metabolized in mixed acid pathway to produce stable acid. The type of acid produced depends on the specific enzymes present within the bacteria. If enough acid is produced, the pH decreases below 4.5 which is indicated by color change of methyl red to yellow. If the bacteria are able to metabolize dextrose present in the broth, it will produce a stable acid which will show color change of methyl red from yellow to red.

Before sterilization, broth was distributed, 6ml each, into test tubes.

A fresh subculture of positive samples was needed for inoculation. The sample was inoculated into MRVP broth using a sterile inoculating loop. The broth was then incubated for 24 hours at 37°C. After incubation, a few drops of methyl red reagent were added to each tube. Color change was observed immediately and results recorded.

Methyl red test has certain limitations:

1. This reaction alone does not produce the results for identification of a particular species.
2. Small amount of inoculums must be used or else growth is inhibited giving invalid results.

2.3.3 Vogues Proskaeur(VP) test:

This test is used to determine an organism's ability to produce acetylmethylcarbinol from glucose fermentation

Before sterilization, broth was distributed, 6 ml each, into separate test tubes.

A fresh subculture of positive samples is needed for inoculation. The sample was inoculated into MRVP broth using a sterile inoculating loop. The broth was then incubated for 48 hours at 37°C. After incubation, 6 drops of alpha-naphthol were added and mixed well for aeration. After aeration, 2 drops of 40% potassium hydroxide was added and mixed well to aerate it. A pink-red color was observed within 30 minutes and results recorded.

Vogues Proskaeur test has certain limitations:

1. Other biochemical tests need to be performed for the identification of particular species.
2. The reagents must be added in order and accurate amounts or weak positive reactions occur.
3. The results must be read within an hour or else a false positive result may be seen.
4. Some organisms destroy acetoin, thus making this test invalid.

2.3.4 MIU (Motility, Indole, Urease) Agar:

MIU agar can be used to carry out three different biochemical tests; motility, indole and urease. The medium contains caesin enzyme hydrosylate as a source of amino acids and other nitrogenous substances. Its sugar source is dextrose and contains phenol red as an indicator. The motility and urea utilization is first observed. An organism that is motile creates a type of diffused growth pattern along the stab line. Organism capable of utilizing urea break it down to produce ammonia which increase pH of medium and causes color of phenol red to change from yellow to pink. Indole is a product of the utilization of tryptophan; an amino acid within caesin enzyme hydrosylate. The indole produced reacts with aldehyde present in Kovac's reagent to form a red complex.

After sterilization, the media was allowed to cool whereupon 5ml of the filtered 40% urea solution was added to 95 ml of the media. The solution was mixed and distributed evenly, 6ml each, into test tubes where they were allowed to cool in an upright position to be stored.

A fresh 24-hour subculture is needed for inoculation. The inoculums were stabbed in the center of the media using a sterile inoculating needed. The tubes were incubated at 37°C for 24 hours. After 24 hours, test results for motility and urease were recorded. Then Kovac's reagent was added and results recorded.

2.3.5 TSI (triple Sugar Iron) test:

TSI media has three sugars present; glucose, lactose and sucrose. It also contains iron which acts as an indicator of H_2S production and contains agar as a solidifying agent. It is a semi-solid medium with a slant and a butt (butt contains higher amount of glucose). It also contains phenol red as an indicator. If an organism is only able to utilize glucose, the production of small amount of acid will turn butt yellow. If an organism is able to utilize all three sugars, the large amount of acid will turn both butt and slant yellow due to decrease in pH. If an organism produces H_2S, it will react with iron in media to produce black precipitate of ferrous sulphide. An organism that produces gas will create bubble or cracks in the media.

Before sterilization, the media was distributed evenly, 6 ml each, into test tubes. After sterilization, the tubes were tilted to create a slant and allowed to cool.

A fresh subculture of inoculums was needed for testing. The inoculums were first stabbed into the butt and then streaked across the slant using a sterile inoculating needle. The tubes were incubated for 24 hours at 37°C. After incubation, the results were recorded.

2.3.6 Glucose Fermentation test:

When an organism ferments glucose it produces an acid or an acid with gas. Depending on which organism acts on it and what substrate is used, several types of end products are produced such as lactic acid, formic acid, acetic acid, etc. Glucose fermentation usually results in lactic acid and production of hydrogen or CO_2. The production of the acid causes the pH in the broth to decrease which cause the color of indicator, phenol red, to change from red to yellow. If gas is produced during fermentation, then a bubble of the gas can be seen within inverted tubes placed in the media known as Durham tubes.

After addition of caesin enzyme hydrosylate, glucose and NaCl, pH was measured to attain a neutral pH of 7.0. After adjustment of pH, phenol red was added and mixed well and then distributed equally, 6 ml each, into test tubes. A Durham tube is added into each test tube to check for the formation of gas. After sterilization, media was allowed to cool.

A fresh subculture is need for inoculation. The inoculums were added to each tube using a sterile inoculating loop and each tube was vortexed to mix well. The tubes were incubated at 37°C for 24 hours. After incubation, the results were recorded.

2.3.7 Nitrate Reduction test:

Nitrate reduction test is to check whether an organism can convert nitrates within the media to nitrites by the enzyme action of nitrate reductase. If the organism has converted nitrate (potassium nitrate) within the medium to nitrites, the nitrites will form nitrous acid. Upon addition of sulfanillic acid, the nitrous acid will react with it to form diazotized sulfanillic acid. This will react with α-naphthylamine to form a red colored compound. The media turning red is indicative of a positive nitrate reduction test.

After sterilization, media was allowed to cool. A fresh subculture of inoculums was needed. Using a sterilized inoculating loop, the sample was inoculated into media and incubated for 24 hours at 37°C. After incubation, a few drops of sulfanillic acid was added which reacts with nitrous acid to form diazotized sulfanillic acid which upon addition of α-naphthylamine forms a red compound indicative of a positive result. Results were recorded.

2.3.8 Catalase test:

This test is used to determine whether an organism produces the enzyme catalase. Catalase releases oxygen from hydrogen peroxide, H_2O_2. The presence of an organism that produces catalase enzyme causes the breakdown of H_2O_2 into water and oxygen which shows the production of bubbles. Lack of bubbles is indicative of a negative test.

A fresh subculture of inoculums was needed. Using a sterile inoculating loop, a drop of sterile saline was placed on a clean glass slide. Inoculums were used to create a suspension on the slide. A few drops of 3% H_2O_2 were added and if organism was catalase positive rapid production of bubbles were seen. Results were recorded.

2.3.9 Gram Staining:

Gram staining method is the most important procedure in Microbiology and was developed by Danish physician Christian Gram in 1884. Gram staining is based on the difference in composition of cell wall of Gram negative and Gram-positive bacteria. Gram positive bacteria have a thick layer of peptidoglycan with cross linking teichoic acid which is able to resist decolorization. In aqueous solutions, crystal violet produces CV+ and CV- ions that penetrate the cell wall of both Gram positive and Gram-negative bacteria. CV+ reacts with negative components of bacterial cell wall to stain the cells purple. Upon addition of iodine, it reacts with

CV+ to form large CV-I complexes within the cytoplasm. The decolorizing agent, ethanol reacts with the lipopolysaccharide layer of Gram negative bacteria causing it to become disintegrated and exposing peptidoglycan layer which is very thin compared to Gram positive bacteria. Ethanol addition causes the peptidoglycan layer to break allowing CV-I complexes to be washed out from cells decolorizing them. However due to thick peptidoglycan layer of Gram positive bacteria, the CV-I complexes within them remain. After decolorization, the counter stain, safranin is added which turns Gram negative bacteria pink.

2g of crystal violet was dissolved in 20ml 95% ethyl alcohol and 0.8g of ammonium oxalate monohydrate was dissolved in 80ml distilled water. The two solutions were mixed to make crystal violet dye.

The powders were measured and grinded using a mortar and pestle. Then they were added to 300ml of water to create Gramm's iodine.

2.5g of safranin was weighed and added to 10ml of 95% ethyl alcohol. It was mixed well and then dissolved in 100ml distilled water to create safranin dye.

Using sterile saline, a smear of inoculums was created on a clean glass slide which was passed over an open flame to set it. Few drops of crystal violet were added to cover the smear. After 40 seconds the dye was washed off. Then Gram's iodine was added, which was also kept for 40 seconds and washed off. Then 95% ethanol was added while tilting the glass slide. Ethanol was added until it is completely decolorized. Then secondary dye, safranin was added. It was kept for 40 seconds and then washed off. The slide was air dried and observed under a microscope.

2.3.10 Kirby-Bauer Disc Diffusion Method:

The purpose of the Kirby Bauer Disk Diffusion method is to determine the sensitivity or resistance of a pathogen organism to various antimicrobial compounds. The pathogenic organisms are grown on Mueller-Hinton agar in the presence of antimicrobial impregnated filter paper discs. The absence or growth of the organisms around the disc determines their sensitivity or resistance to that particular compound. When an antibiotic disc is placed on Mueller-Hinton agar, it absorbs water from the agar and allows diffusion of antibiotic into the agar, thus inhibiting the growth of the bacterial culture if it is sensitive to particular antibiotic.

A bacterial solution of 0.5 McFarland standard suspensions was created and using a sterile cotton swab was dipped into suspensions. The cotton swab was used to create a lawn culture on a Mueller-Hinton agar (MHA) plate. The plate was divided into four divisions and onto each division a single antibiotic disc was added using sterile forceps. The plates were incubated at 37°C for 24 hours. After incubation, the diameter of the clear zones were measured and compared with a standard value to determine the sensitivity or resistance of the sample to that antibiotic**.**

Table 4: List of Antibiotics used for Antibiogram of positive samples of *S. aureus*

No.	Antibiotic name	Abbreviation	Dosage (µg)
1	Nalidixic acid	NA	30
2	Rifampicin	RD	5
3	Tetracycline	TE	30
4	Gentamicin	GEN	10
5	Amoxycillin	AML	10
6	Co-trimoxazole	COT	25
7	Novobiocin	NV	30
8	Levofloxacin	LEV	5
9	Chloramphenicol	C	30
10	Oxacillin	OX	1
11	Erythromycin	E	1
12	Ampicillin	AMP	10

3. RESULTS

3.1 Prevalence of *Staphylococcus aureus* within samples as a percentage:

According to Table 5, 62.5% of fruits and vegetables, that is to say 5 out of 8 samples were positive for *Staphylococcus aureus* , all 4 samples, 100%, of packaged meat was positive for *S. aureus* and 7 out of 18, that is to say 38.9% of milk and its derivatives were found to be contaminated with *S. aureus.*

Table 5: Percentage of positive samples of *S. aureus* in each food group

Type of Sample	Number of positive results for *S. aureus*	Percentage
Fruits and vegetables	5 out of 8	62.5%
Packaged Meat	4 out of 4	100%
Milk and its Products	7 out of 18	38.9%

3.2 Results for Biochemical tests:

Referring to Table 6, it can be seen that *S. aureus* growth on MSA is seen as yellow colonies with yellow zones around them, while other *Staphylococcus* species have no zone color change around them as seen in Figure 6. After observation of samples that have undergone gram staining, it was seen that they appeared as Gram positive, purple cocci in grape-like clusters, as seen in Figures 7a and 7b.

Table 6: Culture characteristics on selective media and Gram staining

Parameter	Results
Culture characteristics on selective media (MSA)	Yellow colonies with yellow zones.
Gram staining	Gram positive cocci in clusters

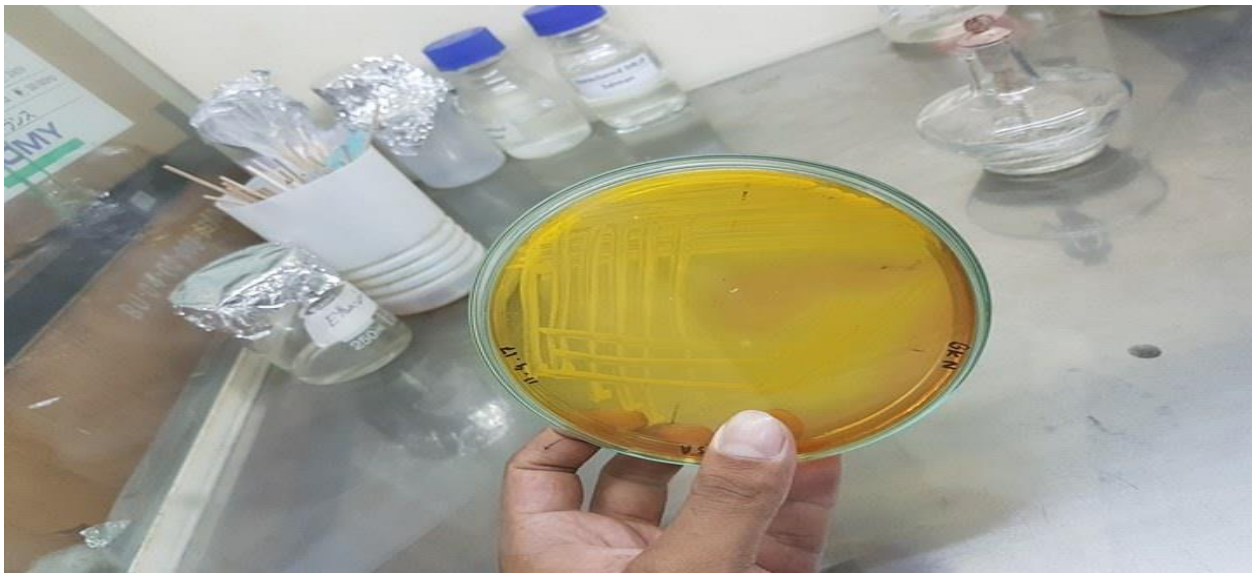

Figure 6: Growth of *S. aureus* on MSA (yellow)

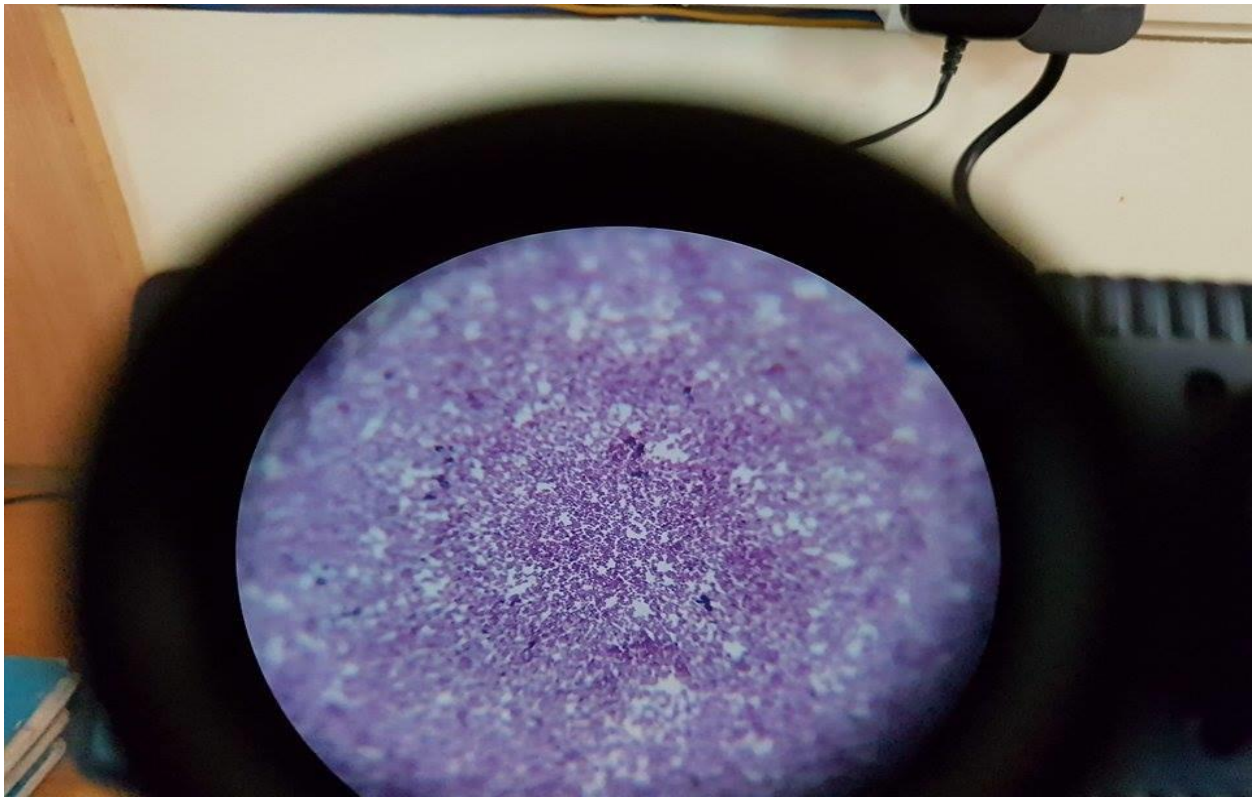

Figure 7a: Observation under microscope of sample PP2 after Gram staining

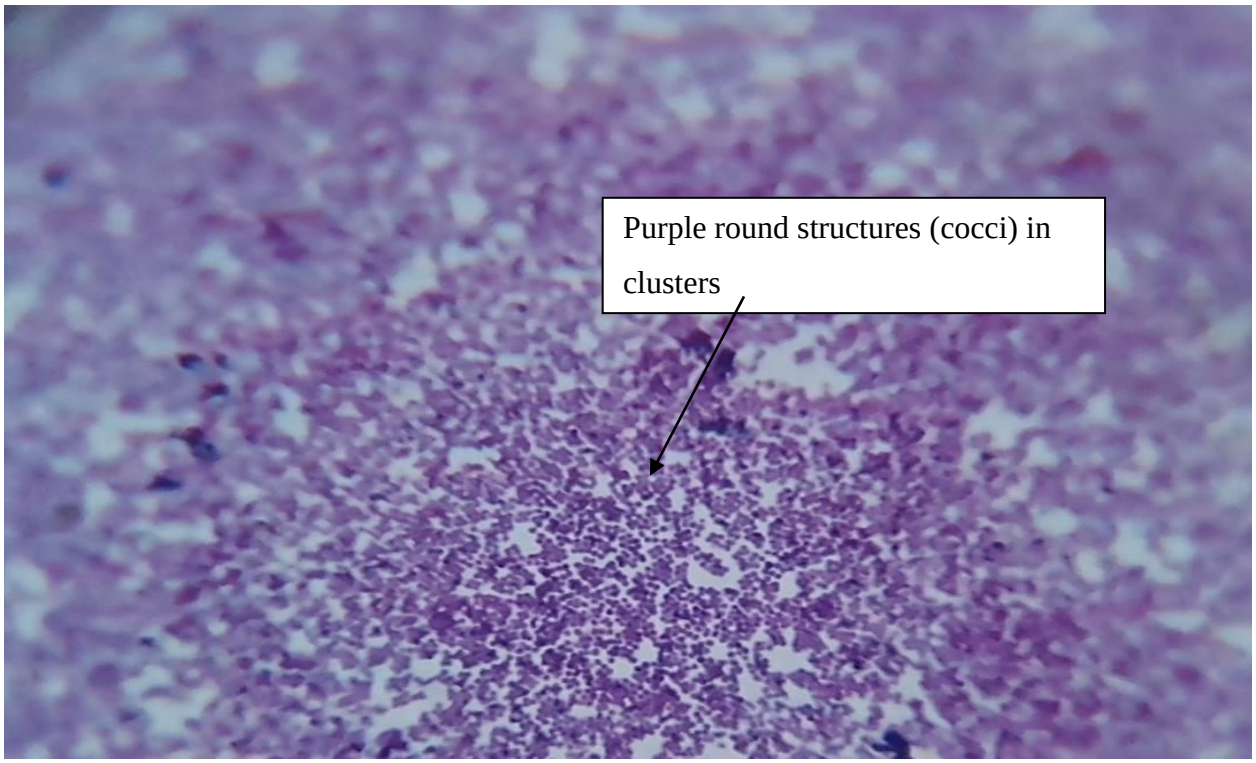

Figure 7b: Closer inspection of same sample showing cocci structures in clusters

As we can see in Table 7, all the organisms test positive for citrate utilization. Therefore, it can be concluded that all these organisms are able to metabolize citrate and break down ammonia salts to produce ammonia which increases alkalinity and changes color of medium from green to blue, as can be seen in Figure 8.

Table 7: Test results of Citrate test

Sample type	Sample number	Result (color)	Inference
Fruits and Vegetables	PP1	Blue	Citrate utilization +
	PP2	Blue	Citrate utilization +
	PP3	Blue	Citrate utilization +
	PP4	Blue	Citrate utilization +
	PP5	Blue	Citrate utilization +
Meat	PM1	Blue	Citrate utilization +
	PM2	Blue	Citrate utilization +
	PM3	Blue	Citrate utilization +

	PM4	Blue	Citrate utilization +
Milk and its products	PY1	Blue	Citrate utilization +
	PY2	Blue	Citrate utilization +
	PY3	Blue	Citrate utilization +
	PY4	Blue	Citrate utilization +
	PY5	Blue	Citrate utilization +
	PY6	Blue	Citrate utilization +
	PY7	Blue	Citrate utilization +

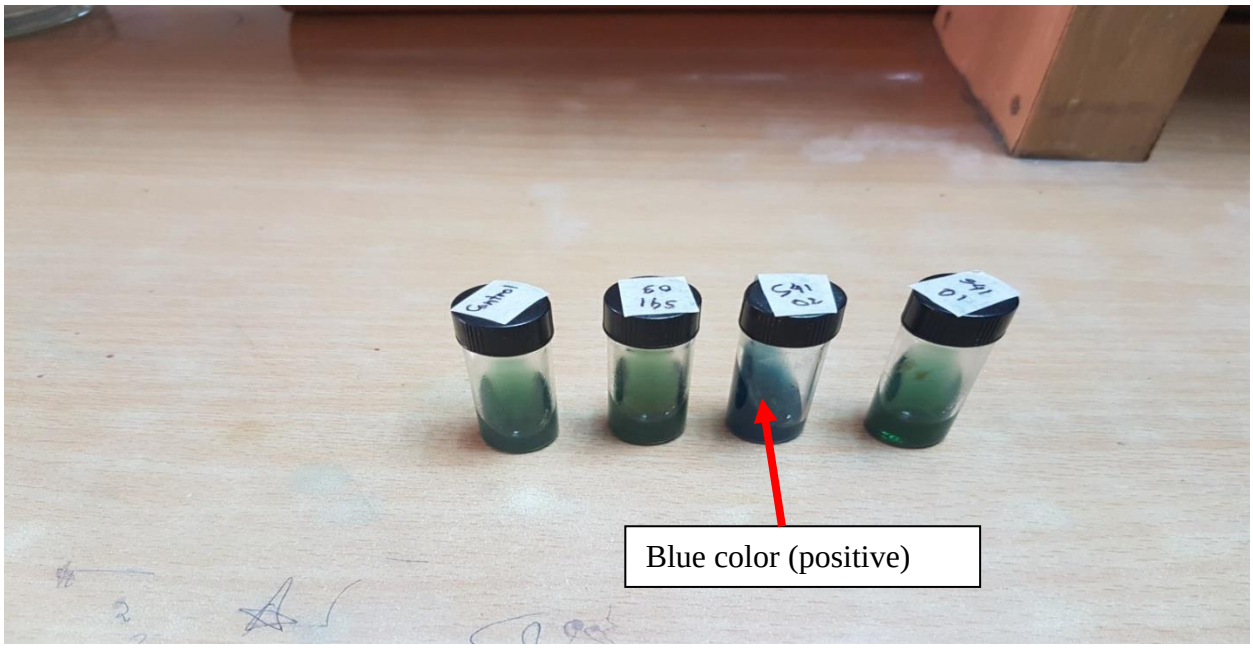

Figure 8: Positive Citrate test result (middle) and negative result (two on the side)

According to the data present in Table 8, we can conclude that all the organism test positive for methyl red test. Thus, the organisms were able to utilize dextrose to form a stable acid which reduced pH of the broth. This in turn changes color of methyl red, upon addition from yellow to red as can be seen in Figure 9.

Table 8: Test results of Methyl red test

Sample type	Sample number	Result(color)	Inference
Fruits and Vegetables	PP1	Red	Acid Production +
	PP2	Red	Acid Production +
	PP3	Red	Acid Production +
	PP4	Red	Acid Production +
	PP5	Red	Acid Production +
Meat	PM1	Red	Acid Production +
	PM2	Red	Acid Production +
	PM3	Red	Acid Production +
	PM4	Red	Acid Production +
Milk and its products	PY1	Red	Acid Production +
	PY2	Red	Acid Production +
	PY3	Red	Acid Production +
	PY4	Red	Acid Production +
	PY5	Red	Acid Production +
	PY6	Red	Acid Production +
	PY7	Red	Acid Production +

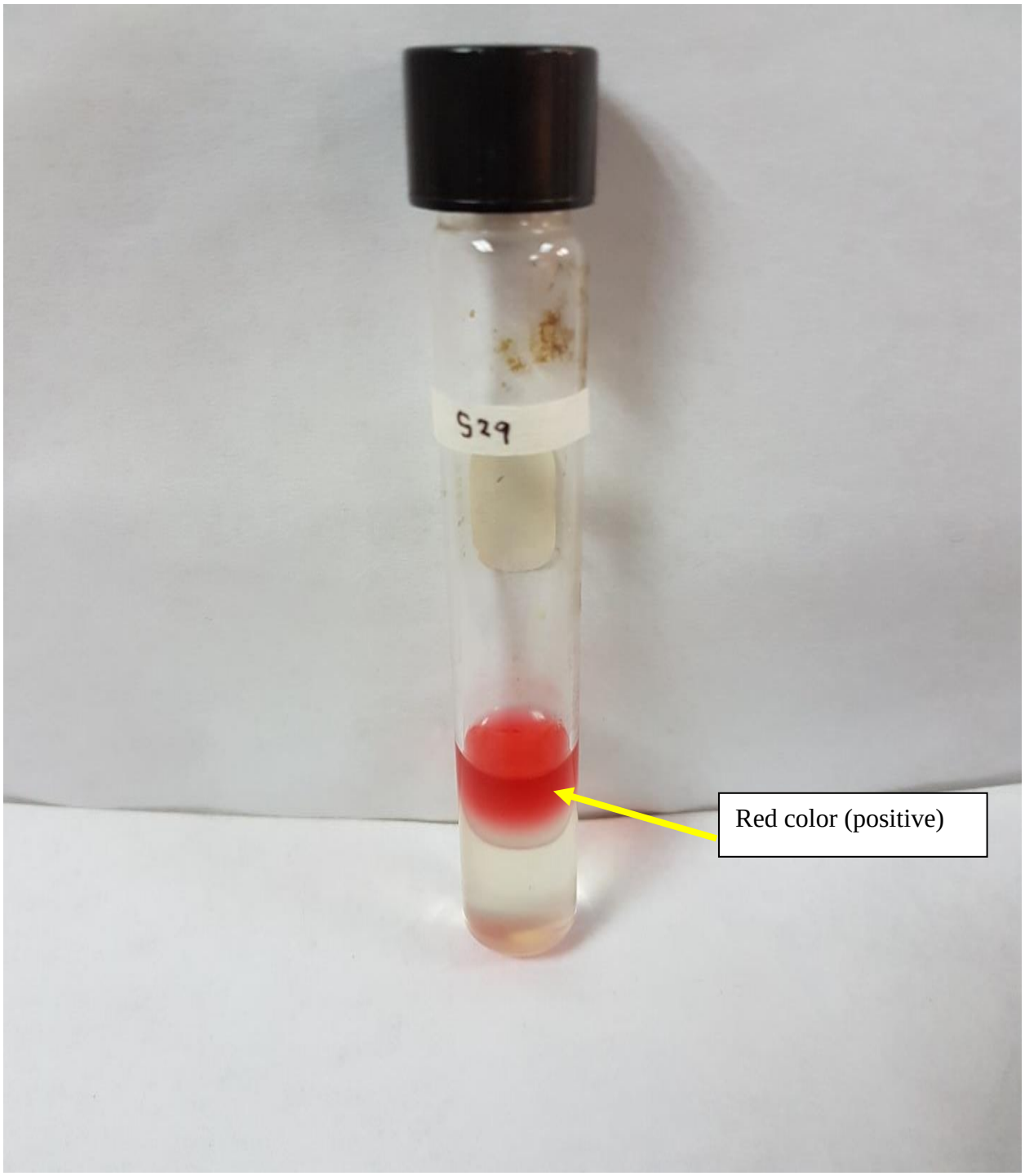

Figure 9: Positive Methyl red test (red color)

Table 9 represents the test results for Vogues Proskaeur test and shows that all samples show positive reaction. All the organisms are able to utilize glucose/dextrose to form acetyl methyl carbine which is converted to diacetyl upon addition of Barritt's reagent A and B which shows a positive red color as shown in Figure 10.

Table 9: Test results of Vogues Proskaeur test

Sample type	Sample number	Result (color)	Inference
Fruits and Vegetables	PP1	Red	Diacetyl formation +
	PP2	Red	Diacetyl formation +
	PP3	Red	Diacetyl formation +
	PP4	Red	Diacetyl formation +
	PP5	Red	Diacetyl formation +
Meat	PM1	Red	Diacetyl formation +
	PM2	Red	Diacetyl formation +
	PM3	Red	Diacetyl formation +
	PM4	Red	Diacetyl formation +
Milk and its products	PY1	Red	Diacetyl formation +
	PY2	Red	Diacetyl formation +
	PY3	Red	Diacetyl formation +
	PY4	Red	Diacetyl formation +
	PY5	Red	Diacetyl formation +
	PY6	Red	Diacetyl formation +
	PY7	Red	Diacetyl formation +

Figure 10: Positive results for VP test

As per Table 10, we can see all the organisms are indole negative, non-motile and urease positive. Therefore, it can be concluded that the organism is not capable of producing tryptophan from casein enzymatic hydrosylate and thus no ring forms at medium interface. Growth is along stab line and there is no diffusion of growth so it is non-motile. Urease was broken down to

ammonia which increases the pH and caused phenol red in media to show color change from yellow to pink. Certain strains were slower to react than other (needed 1 extra hour of incubation). Figure 11 shows MIU agar of PP2.

Table 10: Test results for MIU agar (Indole, motility and urease)

Sample type	Sample number	Indole	Motility	Urease
Fruits and Vegetables	PP1	Negative, no color change	Negative, no growth dispersion	Positive, pink color
	PP2	Negative, no color change	Negative, no growth dispersion	Positive, pink color
	PP3	Negative, no color change	Negative, no growth dispersion	Positive, pink color
	PP4	Negative, no color change	Negative, no growth dispersion	Positive, pink color
	PP5	Negative, no color change	Negative, no growth dispersion	Positive, pink color
Meat	PM1	Negative, no color change	Negative, no growth dispersion	Positive, pink color
	PM2	Negative, no color change	Negative, no growth dispersion	Positive, pink color
	PM3	Negative, no color change	Negative, no growth dispersion	Positive, pink color
	PM4	Negative, no color change	Negative, no growth dispersion	Positive, pink color
Milk and its products	PY1	Negative, no color change	Negative, no growth dispersion	Positive, pink color
	PY2	Negative, no color change	Negative, no growth dispersion	Positive, pink color
	PY3	Negative, no color change	Negative, no growth dispersion	Positive, pink color

	PY4	Negative, no color change	Negative, no growth dispersion	Positive, pink color
	PY5	Negative, no color change	Negative, no growth dispersion	Positive, pink color
	PY6	Negative, no color change	Negative, no growth dispersion	Positive, pink color
	PY7	Negative, no color change	Negative, no growth dispersion	Positive, pink color

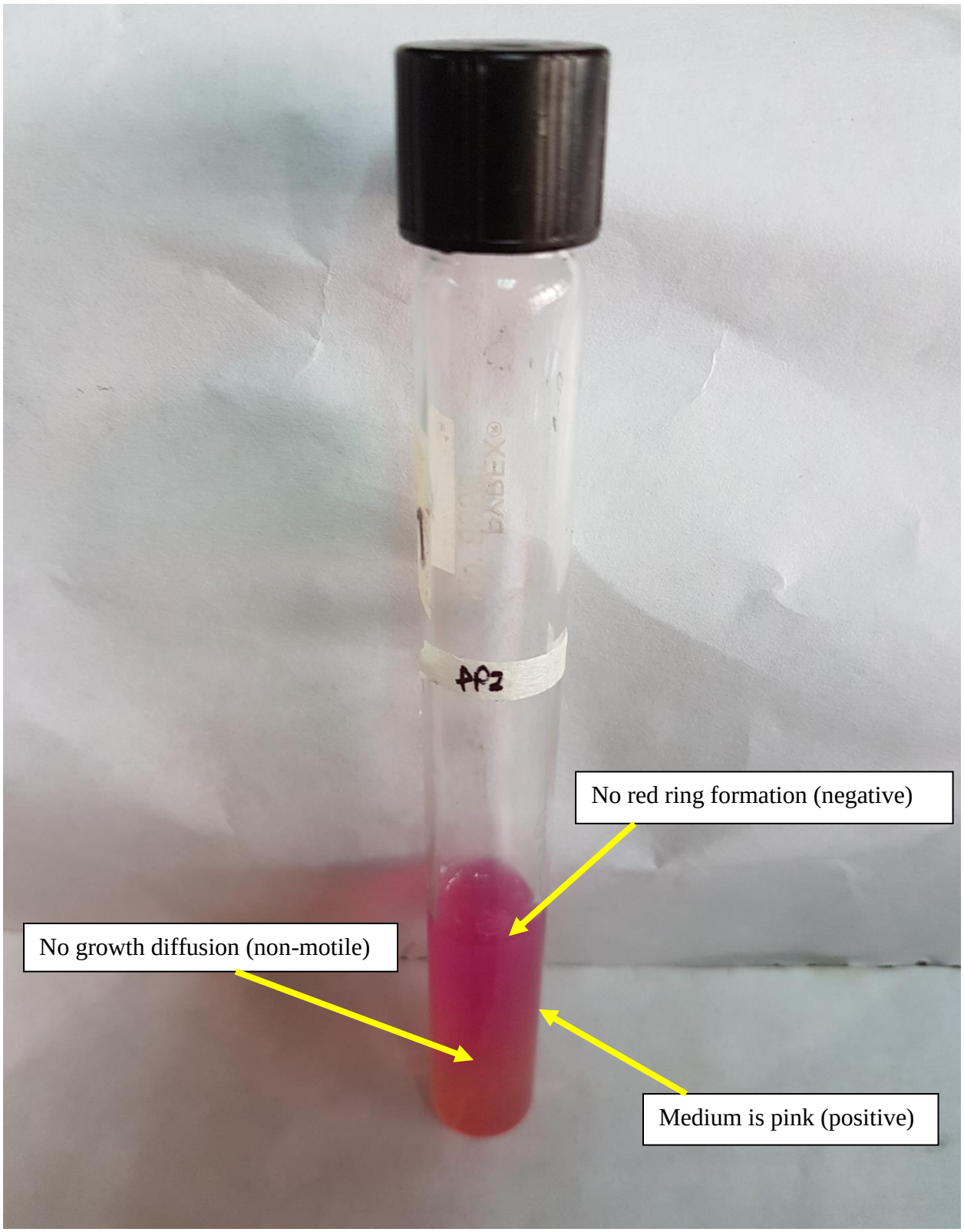

Figure 11: MIU agar of sample PP2

According to table 11, all organisms are able to ferment glucose, sucrose and lactose, as both slant and butt are yellow, which produce acid and reduces pH of medium thus changing phenol red indicator color from red to yellow as shown on Figure 12. There is no gas production and no H_2S production as there is an absence of black precipitate of ferrous sulfide formed by reaction of H_2S with iron within the media.

Table 11: Test results for TSI (Triple Sugar iron) test

Sample type	**Sample number**	**Slant(color)**	**Butt(color)**	**Gas production**	**H_2S production**	**Inference**
Fruits and Vegetables	PP1	Yellow	Yellow	No gas	No black ppt	All sugars fermented
	PP2	Yellow	Yellow	No gas	No black ppt	All sugars fermented
	PP3	Yellow	Yellow	No gas	No black ppt	All sugars fermented
	PP4	Yellow	Yellow	No gas	No black ppt	All sugars fermented
	PP5	Yellow	Yellow	No gas	No black ppt	All sugars fermented
Meat	PM1	Yellow	Yellow	No gas	No black ppt	All sugars fermented
	PM2	Yellow	Yellow	No gas	No black ppt	All sugars fermented
	PM3	Yellow	Yellow	No gas	No black	All

					ppt	sugars fermented
	PM4	Yellow	Yellow	No gas	No black ppt	All sugars fermented
Milk and its products	PY1	Yellow	Yellow	No gas	No black ppt	All sugars fermented
	PY2	Yellow	Yellow	No gas	No black ppt	All sugars fermented
	PY3	Yellow	Yellow	No gas	No black ppt	All sugars fermented
	PY4	Yellow	Yellow	No gas	No black ppt	All sugars fermented
	PY5	Yellow	Yellow	No gas	No black ppt	All sugars fermented
	PY6	Yellow	Yellow	No gas	No black ppt	All sugars fermented
	PY7	Yellow	Yellow	No gas	No black ppt	All sugars fermented

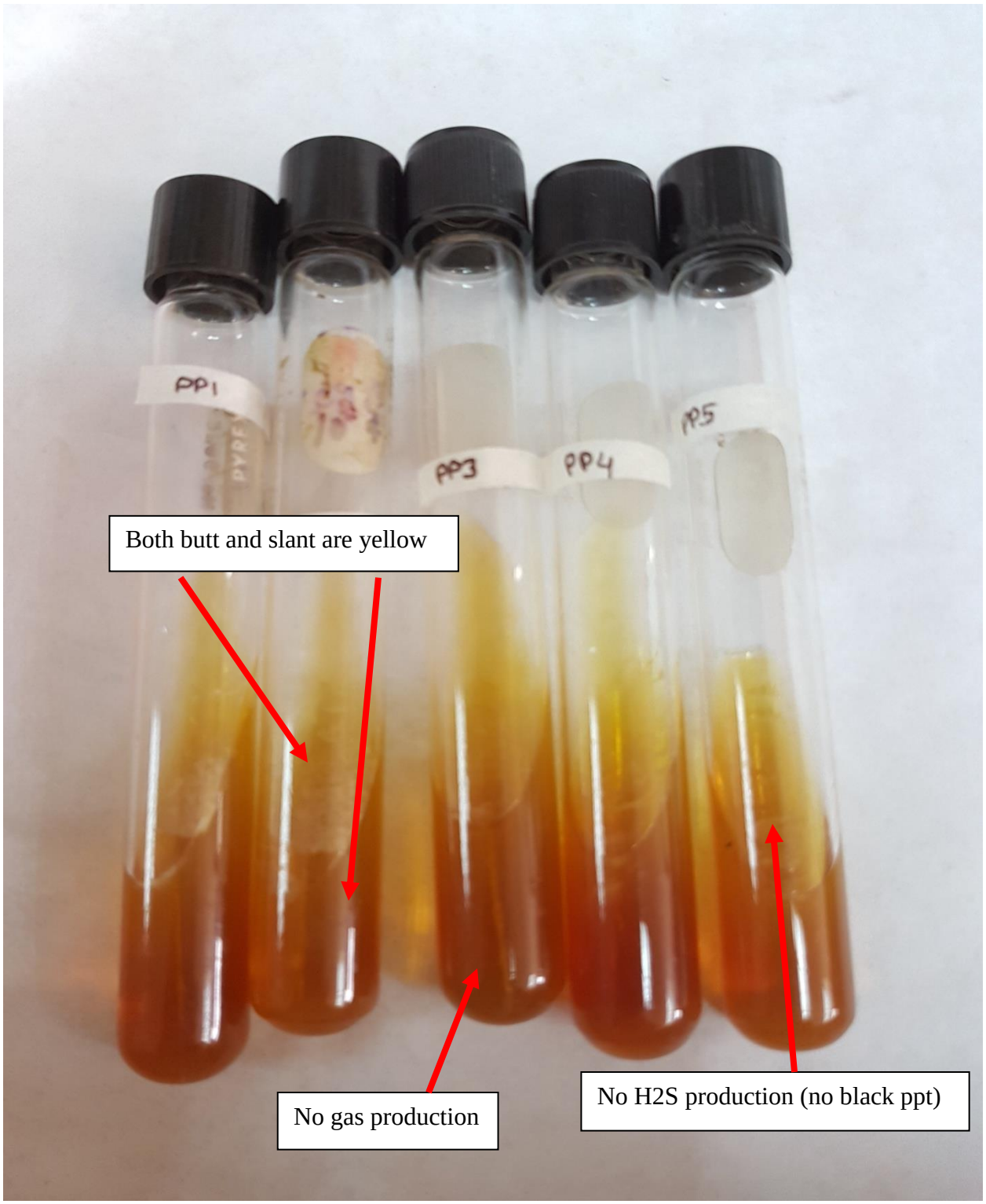

Figure 12: Test results for TSI

As we can see in table 12, all organisms are able to metabolize glucose and therefore test positive for this test. The organism metabolizes glucose to form acid which reduces pH of medium and thus changes phenol red indicator color from red to yellow. The more glucose an organism metabolizes the more yellow the color of broth will be, as shown on Figure 13.

Table 12: Test Results for Glucose fermentation test

Sample type	Sample number	Color change	Gas Production	Inference
Fruits and Vegetables	PP1	Yellow	No gas	Glucose fermentation +
	PP2	Yellow	No gas	Glucose fermentation +
	PP3	Orange	No gas	Glucose fermentation +
	PP4	Orange	No gas	Glucose fermentation +
	PP5	Orange	No gas	Glucose fermentation +
Meat	PM1	Yellow	No gas	Glucose fermentation +
	PM2	Yellow	No gas	Glucose fermentation +
	PM3	Yellow	No gas	Glucose fermentation +
	PM4	Yellow	No gas	Glucose fermentation +
Milk and its products	PY1	Yellow	No gas	Glucose fermentation +
	PY2	Yellow	No gas	Glucose fermentation +
	PY3	Yellow	No gas	Glucose fermentation +
	PY4	Yellow	No gas	Glucose fermentation +
	PY5	Yellow	No gas	Glucose fermentation +
	PY6	Yellow	No gas	Glucose fermentation +
	PY7	Yellow	No gas	Glucose fermentation +

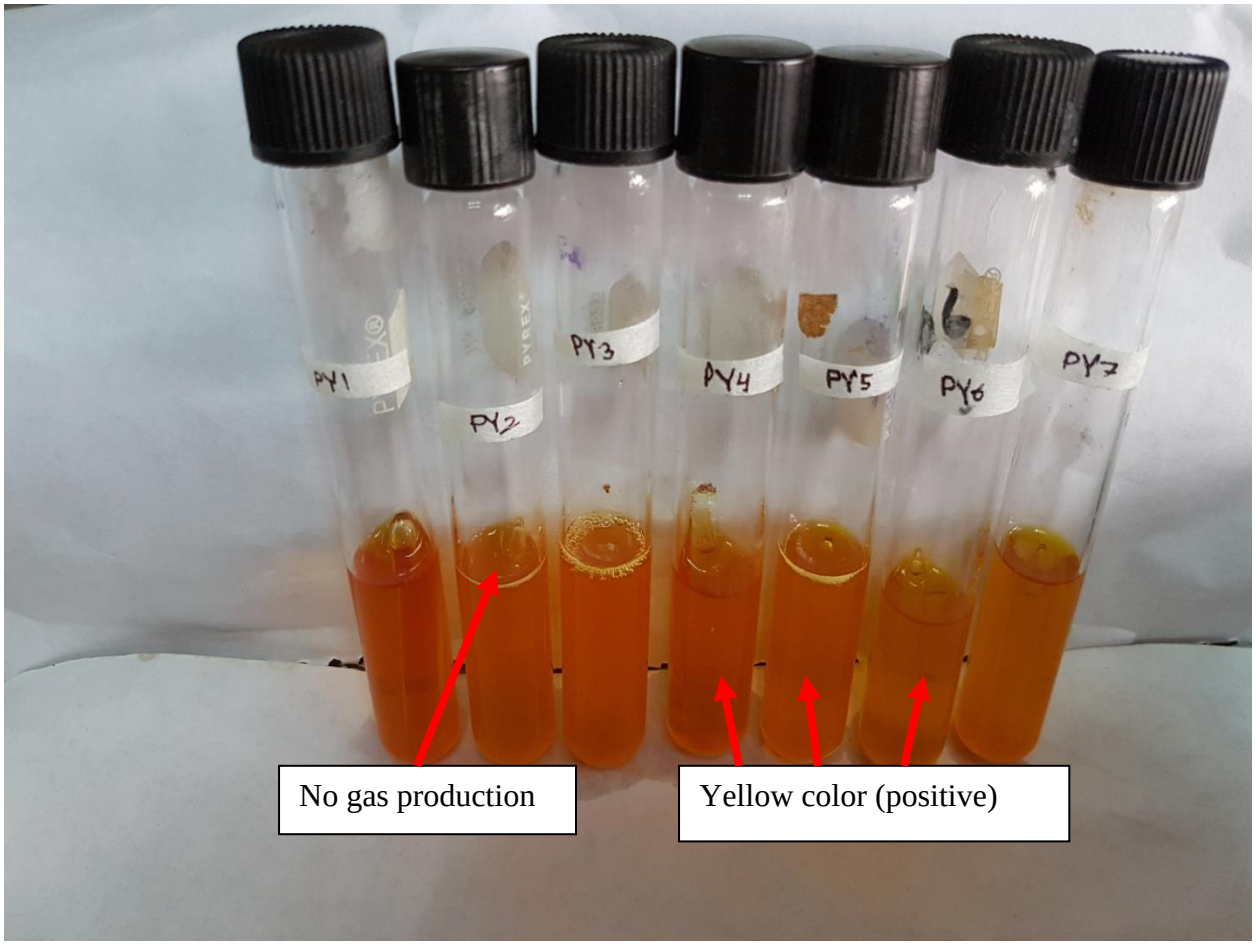

Figure 13: Positive result for Glucose Fermentation test

Table 13 shows Nitrate Reduction test results. The organism shows positive result thus showing they contain nitrate reductase to reduce nitrates to nitrites which forms nitrous acid. Upon addition of sulfanillic acid it reacts with nitrous acid to form diazotized sulfanillic acid which reacts with α-naphthylamine to form a red compound which turns the medium red, indicating a positive result as shown on Figure 14.

Table 13: Test results for Nitrate Reduction

Sample type	Sample number	Result(color)	Inference
Fruits and Vegetables	PP1	Red	Nitrate reduced +
	PP2	Red	Nitrate reduced +
	PP3	Red	Nitrate reduced +
	PP4	Red	Nitrate reduced +
	PP5	Red	Nitrate reduced +
Meat	PM1	Red	Nitrate reduced +
	PM2	Red	Nitrate reduced +
	PM3	Red	Nitrate reduced +
	PM4	Red	Nitrate reduced +
Milk and its products	PY1	Red	Nitrate reduced +
	PY2	Red	Nitrate reduced +
	PY3	Red	Nitrate reduced +
	PY4	Red	Nitrate reduced +
	PY5	Red	Nitrate reduced +
	PY6	Red	Nitrate reduced +
	PY7	Red	Nitrate reduced +

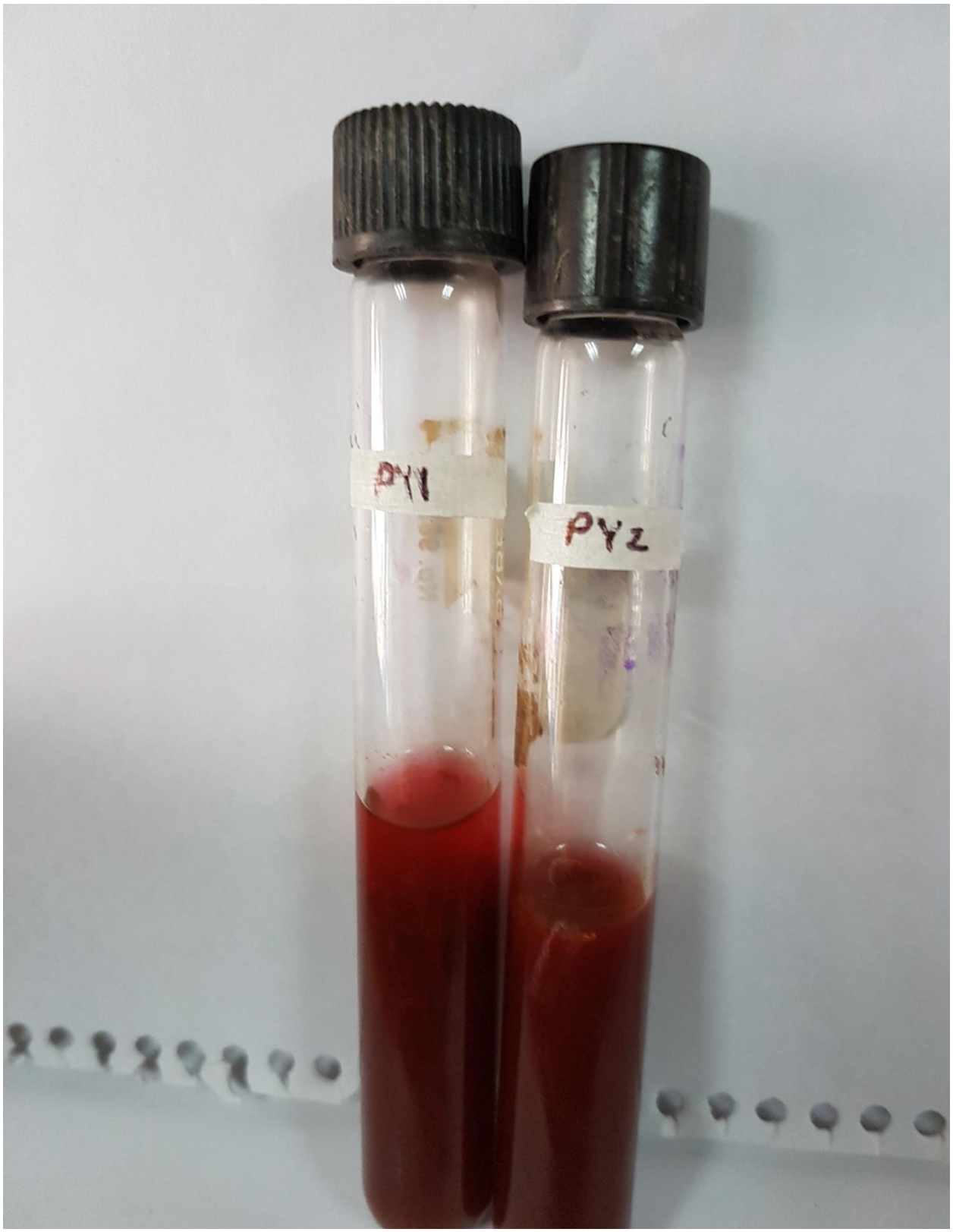

Figure 14: Positive Nitrate Reduction test

Table 14 represents test results for catalase test which indicates whether an organism contains the enzyme catalase. As can be seen, the samples are all catalase positive which means upon addition of 3% H_2O_2, the catalase breaks it down to form water and bubbles of oxygen which can be seen in Figure 15. This also indicates the organisms are facultative anaerobes.

Table 14: Test Results for Catalase test

Sample type	Sample number	Result(bubble formation)	Inference
Fruits and Vegetables	PP1	Bubbles formed	Catalase +
	PP2	Bubbles formed	Catalase +
	PP3	Bubbles formed	Catalase +
	PP4	Bubbles formed	Catalase +
	PP5	Bubbles formed	Catalase +
Meat	PM1	Bubbles formed	Catalase +
	PM2	Bubbles formed	Catalase +
	PM3	Bubbles formed	Catalase +
	PM4	Bubbles formed	Catalase +
Milk and its products	PY1	Bubbles formed	Catalase +
	PY2	Bubbles formed	Catalase +
	PY3	Bubbles formed	Catalase +
	PY4	Bubbles formed	Catalase +
	PY5	Bubbles formed	Catalase +
	PY6	Bubbles formed	Catalase +
	PY7	Bubbles formed	Catalase +

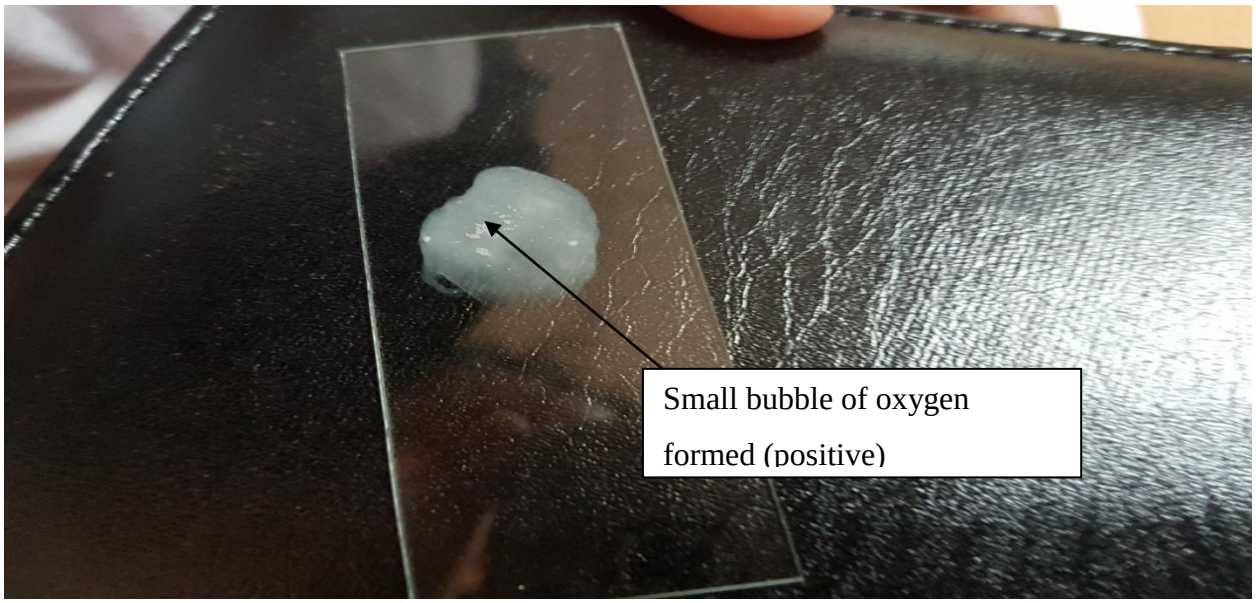

Figure 15: Positive Catalase test

3.3 Test results for Antibiogram:

Table 15, 16 and 17 represent the data recorded from antibiogram. As all isolates were from food samples it can be assumed that most of them are methicillin sensitive *Staphylococcus aureus* (MSSA) and is thus sensitive to most antibiotics. However, a significant (slightly more than half) portion of the isolates are partially resistant, meaning they have grown resistance to said antibiotic or are slowly developing resistance as shown by new growth on clear one. Out of all the isolates, sample PM3 (resistant to 6 antibiotics), PM4 (resistant to 6 antibiotics), PY3 (resistant to 7 antibiotics), PY4 (partially resistant to all antibiotics), PP1 (resistant to 1 antibiotic and partially resistant to 9 antibiotics), PP2 (resistant to 1 antibiotic and partially resistant to 6 antibiotics), PM2 (resistant to 4 antibiotics and partially resistant to 5 antibiotics), PY5 (resistant to 1 antibiotic and partially resistant to 8 antibiotics), PY6 (partially resistant to 10 antibiotics) show a significant level of concern.

Table 15: Antibiogram pattern for *S. aureus* from Fresh Produce

	Sample									
Antibiotics	PP1(mm)	In	PP2(mm)	In	PP3(mm)	In	PP4(mm)	In	PP5(mm)	In
NA	18	R	20	R	21	R	9	R	12*	P
RD	20, 10*	S,P	20, 10*	S,P	14	R	31	S	14	R
TE	21, 10*	S,P	20, 10*	S,P	16	I	30	S	31	S
GEN	28*	P	14	I	25	S	25	S	25	S
AML	28*	P	30*	P	40*	P	27	S	30	S
COT	32*	P	32*	P	15, 5*	I,P	27	S	29	S
NV	37*	P	40	S	36	S	19	S	19, 5*	S,P
LEV	32	S	32	S	23	S	27	S	26	S
C	20, 8*	S,P	17, 5*	I,P	30	S	30	S	30	S
OX	23*	P	20	S	26*	P	16	S	15	S
E	17, 4*	I, P	20, 5*	I,P	28	S	27	S	30	S
AMP	28	I	30	S	42	S	28	S	35	S

Key:

S = Sensitive R = Resistant

I = Intermediate P = Partial resistance

-- = no growth (complete resistance) * = Partial Resistance diameter

In = Inference

Table 16: Antibiogram pattern for *S. aureus* from Packaged Meat

	Sample							
Antibiotics	PM1(mm)	In	PM2(mm)	In	PM3(mm)	In	PM4(mm)	In
NA	21	R	--	R	28	S	26	S
RD	20	S	16, 10*	R, P	--	R	--	R
TE	22	S	20, 10*	S,P	25	S	26	S
GEN	--	R	15*	P	23	S	23	S
AML	30*	P	35*	P	--	R	--	R
COT	20	S	32*	P	30	S	16, 3*	S, P
NV	28	S	27	S	--	R	6	R
LEV	25	S	20	S	33	S	33	S
C	18, 5*	S, P	--	R	30	S	35	S
OX	21*	P	15	S	--	R	--	R
E	20	I	20*	P	12	R	12	R
AMP	32*	P	--	R	--	R	10	R

Key:

S = Sensitive R = Resistant

I = Intermediate P = Partial resistance

-- = no growth (complete resistance) * = Partial Resistance diameter

In = Inference

Table 17: Antibiotic pattern for *S. aureus* from Milk and its products

	Sample													
Antibiotics	PY1 mm	In	PY2 mm	In	PY3 mm	In	PY4 mm	In	PY5 mm	In	PY6 mm	In	PY7 mm	In
NA	17	R	15	R	19	R	15*	R	18*	P	17*	P	15	R
RD	36	S	36	S	--	R	35*	P	13,8*	R,P	17,10*	I,P	30	S
TE	38	S	38	S	19,3*	S,P	37*	P	35	S	33*	P	3	R
GEN	27	S	27	S	19	S	26*	P	17,6*	S, P	16,4*	S,P	20	S
AML	23,3*	S, P	33	S	--	R	21*	P	30,2*	S,P	29,3*	S,P	13	I
COT	26	S	27	S	16,2*	S,P	29*	P	28*	P	27*	P	23	S
NV	38	S	37	S	8	R	36*	P	22,9*	S,P	33	S	3	R
LEV	35	S	29	S	28,4*	S,P	33*	P	26,4*	S,P	25*	P	30	S
C	33	S	33	S	27	S	35*	P	30	S	31	S	32	S
OX	22	S	21,1*	S, P	--	R	20*	P	23*	P	25*	P	20	S
E	12,8*	R, P	21,4*	I, P	8	R	31*	P	24	S	25,3*	S,P	18	I
AMP	26,2*	R, P	38	S	--	R	23*	P	31,5*	S,P	31,4*	S,P	16	R

Key:

S = Sensitive

R = Resistant

I = Intermediate

P = Partial resistance

-- = no growth (complete resistance)

* = Partial Resistance diameter

In = Inference

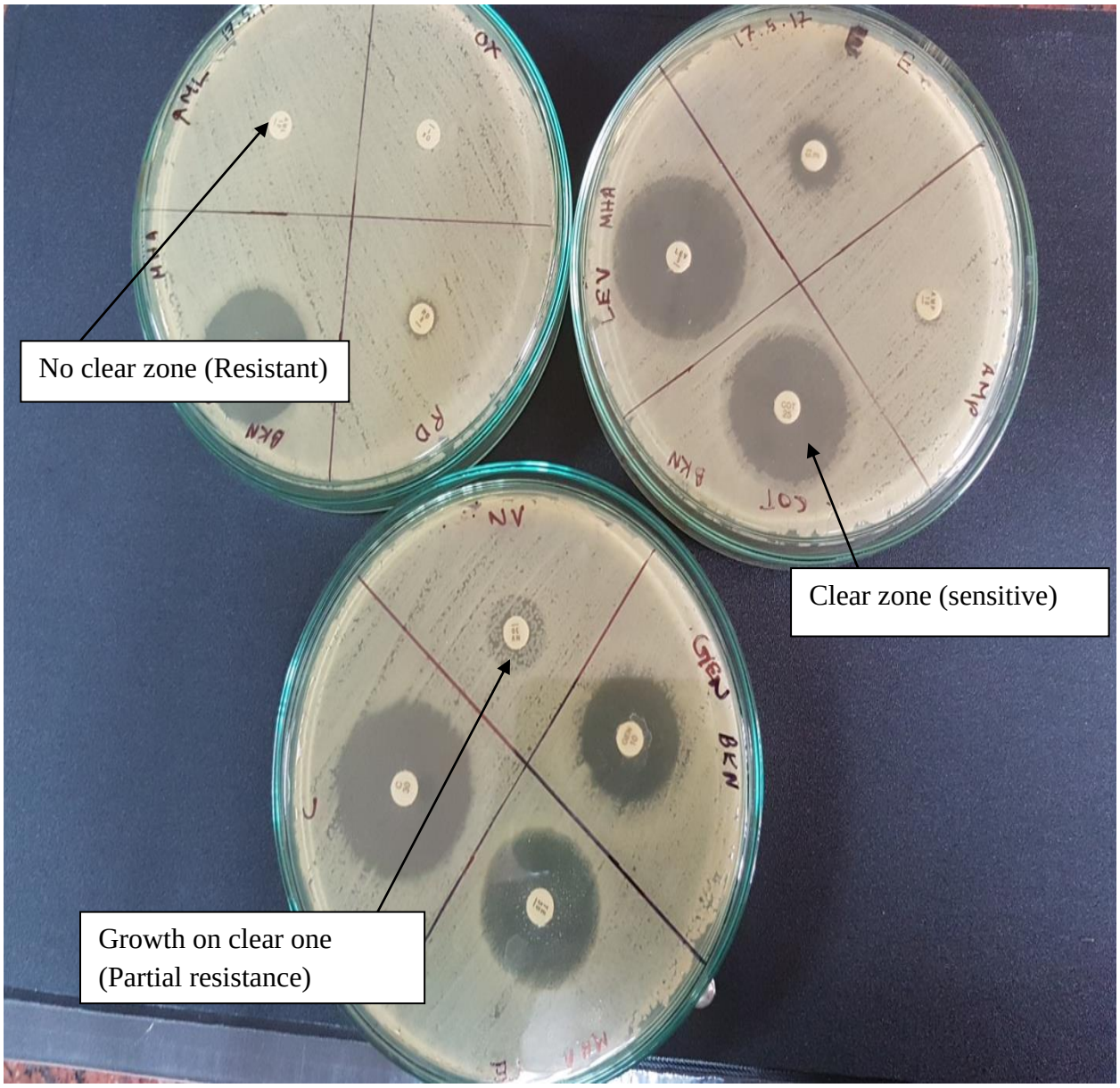

Figure 16: Antibiogram of Sample PM3, (*S. aureus* being tested against 1. Nalidixic acid 2. Rifampicin 3. Tetracycline 4. Gentamicin 5. Amoxycillin 6. Co-trimoxazole 7. Levofloxacin 8. Novobiocin 9. Chloramphenicol 10. Oxacillin 11. Erythromicin 12. Ampicillin)

Table 18: Overall percentage of Antibiotic effect on *S. aureus*

	Overall Percentage			
Antibiotics	**Resistant**	**Sensitive**	**Intermediate**	**Partial Resistance(along with overlapping areas)**
NA	68.75%	12.5%	0	18.75%
RD	43.75%	43.75%	6.25%	37.5%
TE	6.25%	75%	6.25%	37.5%
GEN	6.25%	68.75%	6.25%	31.25%
AML	18.75%	37.5%	6.25%	56.25%
COT	0	56.25%	6.25%	56.25%
NV	25%	62.5%	0	25%
LEV	0	87.75%	0	25%
C	6.25%	81.25%	6.25%	25%
OX	18.75%	43.75%	0	43.75%
E	25%	31.25%	31.25%	43.75%
AMP	37.5%	43.75%	6.25%	25%

Table 18 shows overall percentage of all 16 isolates of *S. aureus* in their resistance, sensitivity or partial resistance against each antibiotic that has been tested. The overall percentage was calculated by dividing the total number of resistant isolated by total number of isolates. The same is true for sensitive and intermediate organisms. The partial resistance was calculated in overlapping areas as well. Certain antibiotic created clear zones which indicated sensitivity but there was a smaller circle showing that the isolate was slowly developing resistance to said antibiotic. At the same time certain bacteria grew over the entire clear zone showing partial resistance is developing. Both these cases were used to calculate the overall percentage of the partial sensitivity graph as shown in Figure 16. Among the antibiotics Levofloxacin showed highest effectiveness with an overall sensitive percentage of 87.75% followed closely by Chloramphenicol at 81.25% and Nalidixic acid showed highest resistance percentage at 68.75%.

Amoxycillin and Co-trimoxazole have highest percent of partial resistance at 56.25% as can be seen in Table 18 and Figure 17.

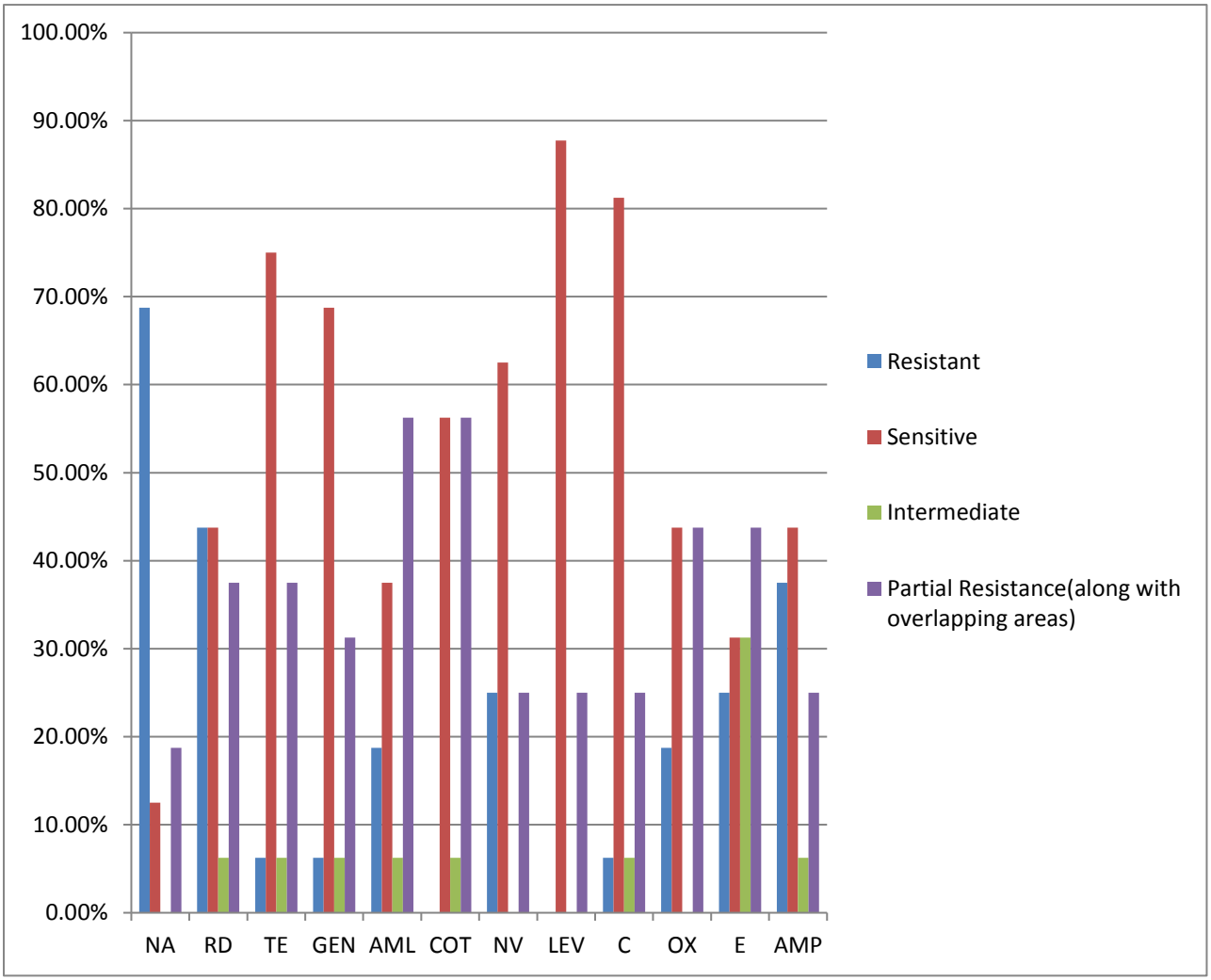

Figure 17: Bar graph of Overall percentage of antibiotic effectiveness against antibiotics used

4.DISCUSSION

4.1 Discussion:

Staphylococcal food poisoning (SFP) is on the most common food borne disease in the world and is usually due to the ingestion of Staphylococcal enterotoxins (SE) (Hennekinne *et al*, 2012). Food borne disease are a global public health problem and their implications are great for health and economy and they cause wide variety of illness and sometime even cause mortality in human beings. The survival of spores through the cooking process, germination, proliferation and production of toxins in food are responsible for human food borne diseases and source of the causative microorganisms (Zewide and Moorthy, 2016). Thus, more hygienic preventive measures are required for reduction of bacterial contamination. The indiscriminate use of antibiotics in therapeutic uses could be the reason for antimicrobial resistance of *Staphylococcus aureus* (Thaker *et al*, 2013).

As per the data of Table 5, we see that 62.5% of fruit and vegetable samples are positive for *Staphylococcus aureus*, 100% samples of packaged meat are positive for *S. aureus* and 38.9 % of milk and its products are positive for *S. aureus*. It is clearly evident that the prevalence of *S. aureus* is highest in packaged meat which is in agreement with the works of Hughes *et al*, 2007, stated that poultry meat was the largest carrier of food borne pathogens followed by red meat. Three out of four packaged meat samples were chicken. Heo *et al*, 2007, reported that *S. aureus* isolated from meat samples showed a resistance of 92.9% and 50% to Tetracyline and Ampicillin respectively which is quite different from the results obtained in this study. It the opposite in fact as 3 out of 4 samples were rather susceptible to Ampicillin rather than Tetracycline. However, as sample size was small, it can still be expanded upon as to whether meat is the most efficient carrier of *S. aureus*.

Fruit and vegetable samples were second highest which state that the handling and storage of these samples in the supermarkets may have been unhygienic (El-Hadedy and El-Nour, 2012). Last was the prevalence of the organism in milk. It had highest number of samples as milk is a favorable medium for most bacteria. Even though contamination was high, 7 samples out of 18, the rate was low, suggesting that extraction, processing, handling as well is storage is decent, at least for several milk products. However, the contaminated samples maybe due to the cow, from where the milk was extracted being infected with *S. aureus* having mastitis; an infection of the udder. It may also be possible that processing or storage was unhygienic but former case seems

more likely as prevalence within milk products is the lowest. The results were in accordance with Thaker *et al*, 2013 and Jahan *et al*, 2015 who state these two reasons as the main concern for the contamination of milk by *S. aureus*.

The results of biochemical tests were as expected and therefore confirmed that isolate was in fact *S. aureus*. As samples were different, each isolate maybe a different strain, so some reacted slower than others and required an extra hour of incubation. The results of the biochemical test proved that it is Gram positive cocci, facultative anaerobe, catalase positive, glucose, lactose and sucrose fermenter, produces no gas or H_2S gas, degrades urease, non-motile, reduces nitrates to nitrite, utilizes citrate, indole negative and methyl red and vogues proskaeur positive (Cappuccino, 2002).

According to tables, 15,16 and 17 it can be seen that most isolates of *S. aureus* are sensitive to most antibiotics used. This was as expected as they are all isolated from food samples. However, 3 isolates, PM3, PM4 showed resistance to 6 different antibiotics in accordance to data of Akbar and Anal, 2013, and PY3 to 7 different antibiotics and partially resistant to 3 other antibiotics. This was an important find as most *S. aureus* samples are expected to be sensitive to all antibiotics. The ones that are resistant maybe due to humans carrying resistant strains of *S. aureus* and contaminating food products with it. This is most likely done by workers within the supermarkets during handling or by the customers. Several hundred customers may pick up same product while deciding which one they want and in doing so may have contaminated the products (El-Hadedy and El-Nour, 2012).

Another isolate, PY4 showed partial resistance to all antibiotics while isolates PP1 showed partial resistance to 9 antibiotics, PM2 showed resistance to 4 antibiotics and partial resistance to 5 antibiotics, PY5 showed partial resistance to 8 antibiotics and PY6 showed partial resistance to 10 antibiotics. This may show that the organism is evolving to grow resistance to these antibiotics. In fact, a lot of isolates from milk samples and fresh produce samples showed growing resistance. This is a very big concern as it may cause diseases in people which are not easily cured.

According to Table 18 and Figure 17, most isolates are still sensitive to antibiotics, especially Levofloxacin (87.75%). However, we can see that a certain number of isolates have grown

partial resistance to every antibiotic tested with highest resistance occurring in Amoxycillin and Co-trimoxazole (56.25%)which is contrary to the data present in Thaker *et al*, 2013.Nalidixic acid was weakest against *S. aureus* isolates with an overall resistance of 68.75%. Even though most isolates *of S. aureus* are known to be sensitive to Novobiocin, certain isolates were seen to evolve to develop partial resistance or complete resistance. While Jahan *et al*, states that 75% of their strains were sensitive to Erythromycin only 31.25% of the isolates in this study were sensitive. This may be due to collection from different sources. The data presented by Akbar and Anal, 2013was in agreement with isolates obtained from meat samples showing that PM3 and PM4 have resistance against Oxacillin and Ampicillin. Staphylococcal food poisoning is a major concern public health and as seen in this study most items taken from a supermarket are contaminated with *S. aureus*. This may be due to contamination of raw materials, contamination during processing or exposure to infected individuals. It is necessary to check procedures and raw materials to ensure it is more hygienic and free of infection. Storage conditions and more hygienic handling by workers and customers must also be monitored (El-Hadedy and El-Nour, 2012).

It has been found that the incidence of food borne pathogens such as *S. aureus* is largely associated with poultry meat and it is a large threat to consumer health. The development of drug resistance by these common pathogens is a matter of concern in food safety. It seems that the majority of commonly used antibiotics are not effective against *S. aureus*. Bio preservation and bio-control strategies should be adopted to cope with the problems related to the chemical preservatives and antibiotics used in animal farming and food processing (Akbar and Anal, 2013).

S. aureus has a wide range of habitats including human body parts, which may contaminate the food. It is considered being one of the most important foodborne illnesses causing pathogenic species. It's present in food indicates poor hygiene and improper storage conditions (Gundogan *et al*, 2005).

Due to indiscriminate use of antibiotics most strains of *S. aureus* have grown or are developing resistance to antibiotics. It is imperative that the resistance pattern of such pathogenic organisms be monitored to ensure that viable treatment is available for anyone infected with this strain. This study was to highlight the most prevalent organism within packaged meat, fruits and vegetables,

milk and its products and to test for its antibiotic sensitivity to check how hygienic storage or handling of food products within supermarkets really are. The purpose was similar to that of Thaker *et al*, 2013.

4.2 Conclusions drawn from this study:

From this study, we have concluded that the selected food groups (packaged meat, milk and its products, fruits and vegetables) were all contaminated with *S. aureus* so it can be said that storage conditions are unhygienic. The biochemical tests confirmed the presence of *S. aureus*. The results of the antibiogram showed slight concern for certain isolates. This is concerning as it alludes to both unhygienic conditions of storage as well as the abuse of antibiotics. To finally conclude this study, it must be said that its content may well serve to raise awareness on both issues and encourage further research in this particular area.

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6. Appendix

Appendix-I

Reagents

1. **0.1% peptone salt solution**

   1g of bacteriological peptone and 8.5g of Sodium chloride dissolved in 1000ml of water.

2. **0.9% saline solution**

   9g of Sodium chloride dissolved in 1000ml of water.

3. **Nutrient Agar (NA)**

   28 of NA dissolved in 1000ml of distilled water.

4. **Mannitol Salt Agar (MSA)**

   111.02g dissolved in 1000ml of distilled water.

5. **Simmons Citrate Agar**

   23g dissolved in 1000ml of distilled water.

6. **MRVP broth**

   7g peptone, 5g dextrose, 5g Potassium phosphate dissolved in 1000ml of distilled water.

7. **40% Urea solution**

   40g dissolved in 100ml of distilled water.

8. **MIU (Motility, Indole, Urease) Agar**

   18g dissolved in 950ml of distilled water

9. **TSI (Triple Sugar, Iron) Agar**

   64.42 g dissolved in 1000ml distilled water.

10. **Glucose fermentation broth**

    1g Caesin Enzyme Hydrosylate, 0.5g glucose, 0.5g Sodium Chloride, 0.0189g phenol red dissolved in 100ml of distilled water.

11. **Nitrate Reduction broth:**

    5g peptone, 3g beef extract, 5g potassium nitrate dissolved in 1000ml of distilled water.

12. **Mueller-Hinton Agar (MHA)**

    38g dissolved in 1000ml distilled water.

13. **Methyl red**

    0.02g of methyl red dissolved in 40 ml ethyl alcohol.

14. Barritt's reagent A

50g of Alpha-naphthol, 5% dissolved in 1000ml absolute ethanol.

15. Barritt's reagent B

40g of Potassium hydroxide dissolved in 1000ml of water.

16. Kovac's reagent

5g of Paradimethylamino-benzaldehye dissolved in 25 ml concentrated Hydrochloric acid and 75ml amyl alcohol.

17. Nitrate Reduction Reagent A

8g of sulfanillic acid dissolved in 1000ml acetic acid, 5N.

18. Nitrate Reduction Reagent B

5g alpha-naphthylamine dissolved in 1000ml acetic acid, 5N.

19. 3% Hydrogen peroxide solution

2.57ml of 35% Hydrogen peroxide diluted with 27.43 ml of distilled water.

20. Crystal violet dye

2g crystal violet dissolved in 20ml 95% ethyl alcohol and 0.8 g of Ammonium oxalate monohydrate dissolved in 80ml distilled water.

21. Gram's Iodine

1g of Iodine and 2g of Potassium iodide dissolved in 300ml of water.

22. 95% ethanol

95ml of absolute ethanol diluted with 5ml distilled water.

23. Safranin dye

2.5g of safranin dissolved in 10ml ethyl alcohol and 100ml of distilled water.

Appendix-II

Instruments

The equipment used for conducting this study are listed below:

Instrument	**Manufacturer**
Autoclave	SAARC
Freeze (-20◦C)	Siemens
Incubator	SAARC
Micropipette (10µl-100µl)	Eppendorf, Germany
Micropipette(100µl-1000µl)	Eppendorf, Germany
Oven, Model: MH6548SR	LG, China
Refrigerator (4◦C) Model: 0636	Samsung
Safety Cabinet Class II Microbiological	SAARC
Vortex Mixture	VWR International
Weighing Balance	ADAM EQUIPMENT^{TM}, United Kingdom